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BIOLOGICAL BULLETIN

A CONTRIBUTION TO OUR KNOWLEDGE OF THE FUNCTION OF THE ŒNOCYTES OF INSECTS.[1]

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The function of the cells known as the œnocytes has been the object of a considerable amount of speculation by various investigators. The studies which constitute the basis of these speculations have all been of a morphological character and, while valuable in their way, throw no light on the physiology of these very singular elements. So much has been written about them, especially about their morphology, that we will consider only a few of the more interesting views on their possible function. In 1873 Graber called attention to the fact that the œnocytes are glands secreting a substance concerning which nothing is known. Later ('91) he supposed that they are metamorphosed into the fat-body, and also give rise to blood corpuscles. This was corrected by Wheeler, in 1891, who through a study of the embryological development of these cells, concluded that they neither give rise to the fat-body nor to the blood. Pantel ('98) and Berlese ('99) endowed the œnocytes with an excretory function, the latter supposing that they serve during the periods of moulting and pupation when the Malpighian tubules are functionless. Anglas ('00) advanced the view that the œnocytes may possibly secrete ferments. Koschevnikov ('00) makes the remarkable statement, among others, that he has preparations which show plainly that the œnocytes swallow fat cells. He further says, "unnecessary substances which get into the blood stop in the interior of the œnocytes in the form of granules. The cells are not periodically emptied of this excretion product, but

[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University. No. 57.

are continually being filled. Finally they are completely filled and are made entirely useless for further activity. It may be that the inability of the œnocytes for further work brings about a disturbance of the regular metabolism, and is hence one of the causes of the sinking life activity of the insect." It seems as if Koschevnikov must have confused the œnocytes with phagocytes; at any rate he did not study the œnocytes. Janet ('07) regards the œnocytes as real unicellular glands. "Like these they take from the blood elements for their functional activity. The substance resulting from this activity they give up by osmosis to the adipocytes which use it possibly for the elaboration or dissolution of reserves, possibly for the production of a special reserve substance." Verson ('11) found at times at the periphery of the cells microscopical exudations of a particular excretion which was accompanied by changes in the form and size of the nucleus. Gee ('11) injected specimens of *Platyphylax designatus* larvæ with methylene blue, and found that immediately after injection the œnocytes and spinning glands both took the stain, the œnocytes less than the spinning glands. "The Malpighian tubules, except in one case, did not take the stain. In larvæ killed half an hour after injection the œnocytes were more deeply stained, but no coloration was observed in the Malpighian tubules. Larvæ killed one hour after injection showed that the Malpighian tubes had begun to take up the blue color, but that the œnocytes and spinning glands were becoming less intense, in coloration." He reached the conclusion that the similar reaction of œnocytes and spinning glands towards methylene blue seems to show that the œnocytes are secretory rather than excretory, the spinning glands being secretory. "The nature of their secretion is difficult and practically impossible to determine. Can it be that their function is the secretion of a substance or enzyme which is of aid to the fat-body in its constructive work?"

It will be seen from this short review that there are many and diverse opinions as to the function of the œnocytes. On reviewing the literature on the morphology of these cells, the divergence of views is seen to be still greater, no two people agreeing in more than a few points. The insects used by the

various investigators were different species and very often insects belonging to distantly related orders. Of course, the form and body orientation of the œnocytes is quite different in non-related forms and even in members of the same species at different periods of their life history. This is exactly what we should expect to find. In numerous cases, however, the varied opinions can be attributed to the fact that many of the investigators did not study the same cells.

I have made sections of larvæ of Trichoptera, Lepidoptera and Diptera and find that the differences between their œnocytes are merely differences of size, shape, density of granulation and amount of ramification of the nuclei. Generally speaking the œnocytes are large, yellow, more or less isolated cells, so large in fact that in some forms they can be readily identified with a pocket lens. They are located in the abdominal segments and in such only as bear spiracles. Here the œnocytes are situated behind the tracheæ. They do not seem to be definitely attached to the tracheæ and sections do not reveal the intrusion of tracheal filaments into these cells. A cytological study with the orange G and iron hæmatoxylin method shows the cytoplasm to be finely granular and the nucleus to be greatly enlarged and ramified, giving the cells the appearance of being highly active.

To throw any light on the physiology of these cells was a difficult task. Comparatively speaking, they are large, yet in nearly all forms too small to deal with experimentally. To be certain of any test one has to have a bulk of material and further after obtaining it, one must be able to dissect out the organs with ease and be certain at all times that they are the same. Fortunately Mr. James W. Chapman, entomologist of the city of Boston, called my attention to the larvæ of the leopard moth (*Zeuzera pyrina*). The life history of this caterpillar, as worked out by Mr. Chapman, extends over a period of three years, during which time it feeds on practically every species of tree or shrub except conifers, and attains at the end of the third year a very large size, accumulating a great amount of fatty tissue and consequently becoming very heavy. On dissecting some of these larvæ, I found the œnocytes to be so enormous that in a three-year-old larva, a cluster could be recognized with the naked

eye. In one and two year old larvæ they are much smaller, for they grow in size with age as do the other organs. In this species, the œnocytes are located in the seven abdominal segments which are just those which bear the abdominal spiracles. Behind these they are situated, occurring in clusters around the tracheæ. There are only two clusters to a segment, one on each side. The number of cells in a cluster varies, from ten to forty or fifty, in different segments. The size also varies considerably in different segments (diameter 175–250 μ) and even in the same cluster, yet the smallest one far surpasses the size of any other cell within the body of the insect.

Through the kindness of Mr. Chapman, I was able to obtain a large amount of material and, following the suggestion of some of the previous investigators, principally Anglas and Janet, I began to work on the hypothesis that the œnocytes are unicellular glands, perhaps secreting a ferment.

A number of tests were made for lipase and oxydase. First, however, to become thoroughly acquainted with the reactions of these enzymes, the pancreases of four hogs were used. Lipase and oxydase are known to occur in these organs, and it was thought advisable to work with them for a time before applying tests to the œnocytes. The pancreas was cut from the hog immediately after it was slaughtered, so as to be certain that it was quite fresh. An extract of the organ was at once preserved in toluol to keep out all bacterial infection. This extract was then diluted with physiological .65 per cent. salt solution, one part of extract to ten parts of saline solution. To two cubic centimeters of this diluted extract one fourth of a cubic centimeter of ethyl butyrate was added, plus a small quantity of lacmoid solution. Purified lacmoid crystals were used and the solution was made as nearly neutral as possible. The extract was, of course, kept under the layer of toluol while the other reagents were being added. The specimen was then put in an incubator at body temperature and kept there for 24 hours. As a control test a second and aliquot portion of pancreatic juice was boiled in order to kill any enzyme. This was treated with the same reagents in exactly the same manner, and put in an incubator for 24 hours. At the end of this time both specimens

were taken out and it was found that the liquid in the test tube containing the live enzyme had turned red. The fat-splitting enzyme (lipase) had split the ethyl butyrate into alcohol and butyric acid. The control test retained its former blue tint, namely, that of the lacmoid.

The next thing was to see with how small a quantity of extract and reagents the reaction could be obtained. For this purpose very small glass tubes of equal sizes were blown. For the liquids eye droppers of equal sizes were used. A drop was sucked into the dropper and the glass was graded into four equal parts, so that it was possible by having droppers of exactly the same size and gradation to use for each test the same amounts of liquid. The amounts of the substances were then decreased until two drops of pancreatic extract and one fourth of the amount of one drop of ethyl butyrate plus the lacmoid solution were used. The toluol was never omitted. Control tests were again made each time. The characteristic red color was obtained.

When I was thoroughly satisfied that I had mastered the reaction, I made an extract from the œnocytes of large leopard moth larvæ. Two caterpillars, each measuring about 1¾ inches, were used for each experiment. All of the œnocytes were dissected out in physiological salt solution and rubbed in a very small agate mortar. The extract from the œnocytes plus a small quantity of saline solution which was added equalled two drops. This was taken without any further dilution and, accompanied by control tests, treated in exactly the same way as the small amounts of pancreatic extract had been treated. Six experiments were performed. In not one case did I get an acid reaction. This seems to indicate that lipase or a fat-splitting enzyme is not present in the œnocytes.

It occurred to me that perhaps the fat of insects might be different from vertebrate fat, and that after all the œnocytes might secrete a ferment of some sort, the presence of which the above reagents would not reveal and which might have the power of splitting this fat. I could find in the literature on fats, nothing but the broad statement that all animal fats are triglycerides of oleic, stearic and palmitic acids. The important question for me was therefore to determine whether or not insect

fats are triglycerides. This was easily accomplished by making the acrolein test. Fatty tissue was dissected out of larvæ and crushed and the fat extracted with ether. This was filtered and evaporated. An amount of the evaporated fat was then heated gently with some potassium acid sulphate till vapors appeared. These were smelled and the nasty odor of acrolein was at once detected. Control tests were made with lard. Hence, I think, I am justified in saying that insect fats are like other animal fats, and further think it safe to say that since insect fats are triglycerides like vertebrate fats, a fat-splitting enzyme like lipase would react to the reagents used for determining that enzyme in vertebrate fats. It must also be considered that a simple ester like ethyl butyrate is chosen for the test on account of the ease with which even the weakest lipase will decompose it. It is therefore quite reasonable to say that lipases are absent when ethyl butyrate is not acted upon.

The tests for oxidizing enzymes were far more difficult, but in the end the results were positive after the technique had been perfected.

The ordinary way of determining whether one is dealing with a peroxydase or a true oxidizing ferment is by the guajacum tincture method. This method, although repeated trials were made, gave no results so far as the œnocytes were concerned. The guajacum was never decomposed and the H_2O_2 seemed not to be acted upon. As will be seen later, the œnocyte extract really decomposed the H_2O_2, but as I had no efficient indicator, it was practically impossible to tell whether there was any reaction, although the amount of the reagent was decreased in proportion to the amount of the extract. When large amounts of extract from whole caterpillars were used, I found, as did Ostwald ('07), that very often the H_2O_2 was decomposed so violently as to cause the liquid to bubble up in the test tube. When using such large amounts of extract the guajacum is also acted upon and the "Hartzsuspension" turns blue, showing peroxydases to be present also.

The method I applied to the œnocytes and which I am about to describe, gave positive results in so far as it showed that the œnocyte extract contains oxidizing ferments to a much greater

degree than any of the other organs or tissues. I was unable to classify these oxidizing enzymes any further and am unable to say whether they are oxydases or katalases. A very delicate method had to be devised, for one must bear in mind that the amount of œnocyte extract which can be obtained even from a large number of larvæ is very small. I have already called attention to the fact that the œnocytes are very large in *Zeuzera pyrina* when compared with those of other forms, yet this statement is merely relative and it must be remembered that from the point of view of the physiological chemist they are really minute organs.

The pancreatic extract of the hog was again used and the amounts of the extract and reagents were again decreased in order to train the eye as before to light reactions. Training the eye was hardly necessary in either case. The reaction for lipase was decidedly negative while the reaction for oxidizing ferments was decidedly positive. It was thought safer, however, to take these precautions.

It might be well here to say something concerning the reagent, which was that employed by clinical workers for the demonstration of occult blood. This reagent consists of: 100 c.c. of a 20 per cent. solution of NaOH + 2 grs. phenolphthal*ein* + 10 grs. zinc dust. This is boiled slowly till the solution is decolorized. The fluid is then filtered while still hot into a colored bottle under white petroleum oil. Great care must be exercised to keep out the oxygen of the air, or it will color.

In the experiments one half the quantity of reagent was added to double the quantity of pancreatic extract and a drop or two of a 3 per cent. solution of hydrogen peroxide was added to this. Owing to the fact that oxidizing enyzmes are present in the pancreas, the solution strikes a red color. As control tests water was used instead of extract and treated in the same way. No attention was paid to change of color which develops on prolonged standing. A layer of petroleum was always kept above the specimens to exclude the air.

It was found that the above reagent was satisfactory only so long as a considerable amount of extract was used. When I came down to using very small amounts, *e. g.*, one or two drops,

the alkali was entirely too strong and prevented the appearance of a red color. Hence, it proved to be necessary to resort to a finer reagent. The phenolphtha*lin* had first to be isolated as such. A quantity of reagent for occult blood was taken, hydrochloric acid was added till the precipitate that formed had redissolved. The solution was extracted with ether which was washed 3–4 times with small quantities of water to get rid of the acid. The ether was then evaporated without heat in hydrogen. The evaporation in an indifferent gas is safer, although not absolutely necessary, for I find that the phenolphtha*lin* crystals will keep splendidly if stored in very small vials especially when they are filled to the brim before corking so as to exclude the air. Both methods were tried, however. On evaporation one obtains a crust of phenolphtha*lin* which may, if desired, be crystallized from alcohol or a mixture of ether and petroleum ether, which should evaporate spontaneously.

A small piece of phenolphtha*lin* was now placed in a drop of pancreatic extract to which a drop of a ½ per cent. KOH solution and a drop or two of a 3 per cent. solution of hydrogen peroxide had been added. All work was done under oil. The characteristic red color was immediately obtained. Control tests with water were performed each time.

The great quantity of KOH ordinarily used in making the reagent for occult blood is necessary to reduce the phenolphthal*ein* to phenolphtha*lin*. But after this is accomplished the excess of KOH must be gotten rid of as described above, otherwise the color will not develop when such infinitesimal quantities of extract have to be dealt with. A drop or two of ½ per cent. KOH solution seems to be just the proper amount and concentration to obtain the red salt.

In the last experiment with the hog extract two drops of extract were used; a crystal of phenolphtha*lin* was added and to this one drop of ½ per cent. KOH solution plus one drop of hydrogen peroxide. The characteristic red color was immediately obtained. I repeat, as this is very important, that all work was done under oil. Control tests likewise accompanied the actual tests.

Two drops of œnocyte extract were now taken, treated in

exactly the same manner as the small amounts of hog extract had been treated, and the immediate reaction was very characteristic. Eighteen experiments of this sort were performed and as all cells have catalytic ferments, other cells as the fat cells, intestinal cells, etc., were submitted to the same tests. When very small amounts of the extract from these cells (*i. e.*, amounts proportional to those of œnocyte extract) were used, it was impossible to determine whether a pink color had developed or not.

Conclusion.

I conclude from the preceding experiments that the œnocytes, which have been regarded by previous investigators as glands, secrete oxidizing enzymes. I do not know whether this is their only function, but it is certainly one of them. At any rate they do not secrete a fat-splitting enzyme. Since these cells, hanging loosely to the tracheæ, lie free in the blood, the enzymes which they secrete may activate the oxygen of the body towards combustion. That the cells actually secrete is indicated by the fact that numerous observers, myself included, have detected microscopical exudations around the periphery of the cytoplasm, especially at times when the nucleus is greatly ramified, and therefore manifesting its great activity.

Exactly what relation the œnocytes bear to the tracheæ, I am unable to say. I saw no definite attachment, but am inclined to believe that a relation exists and that through this the oxydases, one branch of the group of oxidizing enzymes, are able to get their molecular oxygen with the formation of peroxides. It must, moreover, be remembered that Wheeler, in 1892, found the œnocytes of phryganeid larvæ to be provided with delicate processes which are attached to the tracheal hypodermis. That may, of course, be simply a means for attachment and have nothing to do with the passage of oxygen from one to the other. The location of the œnocytes may be purely due to the absence of certain mechanical forces. Wheeler found that "the œnocytes originate by delamination or immigration from the ectoderm, just caudad to the tracheal involutions and after their differentiation from the primitive ectoderm never divide, but gradually increase in size." Since they never divide after differentiation, the me-

chanical forces of cell division being absent, they are not able to be carried far from their starting point. Moreover, since there are no other growing tissues between them and the ectoderm, except a few small muscles and connective tissue, they are not pressed or shoved out of the way very much, and are so able to retain their original position. I am inclined, however, to the former view, that there is a definite functional relation between these glandular cells and the tracheæ.

That the œnocytes in *Zeuzera pyrina* are so large is probably due to the prolonged larval stage of this insect. As previously stated, it remains a larva three years, during which time it eats ravenously, and grows very heavy, acquiring an enormous amount of fat. Naturally, since it eats so much, and stores up so much reserve food, it has a great deal of material to oxidize, and consequently needs a large supply of oxidizing enzymes. A comparison with other insects would lead me to this view, for those having a short larval stage like the Dipteran larvæ, have much smaller œnocytes in comparison with the remainder of the body than forms with a prolonged larval life, like the phryganeid larvæ, for example.

Before closing, I wish to express my thanks to Professor William M. Wheeler for the kindly advice and encouragement which he has given me at all times. I also wish to thank my father, Dr. Charles Glaser, of Baltimore, for many valuable technical points.

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UPON THE FORMATION OF HYDROCHLORIC ACID IN THE FOVEOLÆ AND ON THE SURFACE OF THE GASTRIC MUCOUS MEMBRANE AND THE NON-ACID CHARACTER OF THE CONTENTS OF GLAND CELLS AND LUMINA.[1]

B. C. H. HARVEY AND R. R. BENSLEY.

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INTRODUCTION.

Ever since it was discovered that hydrochloric acid was produced by the stomach, investigators have been interested in determining the mode and place of its formation. Is this acid produced as such by the gastric glands and even by particular cells of these glands, or do the glands produce only chemical substances which are not themselves acid but which, interacting in the foveolæ or on the surface of the mucous membrane, produce there for the first time the acid as such?

Among those who have sought to discover the origin of this acid, Claude Bernard, Brücke, Lepine, Trinkler, Gmelin and Oppel were unable to find it definitely localized in the glands.

The results of our investigation demonstrate that only non-acid substances are formed by the glands, and that the contents of the gland cells and lumina are not acid, even when hydro-

[1] From the Hull Laboratory of Anatomy, University of Chicago.

chloric acid is being actively formed on the surface of the mucous membrane.

In the first part of this paper we shall examine very briefly the evidence which has led some to think that free hydrochloric acid is formed in the parietal cells. We shall consider especially the Prussian blue reaction, which, as used by Miss Fitzgerald ('10), has given by far the most definite results. We shall report some results which we have obtained by this method, which in connection with those reported by her show that this reaction does not prove the presence of free hydrochloric acid in the gastric glands under normal conditions.

In the second part of the paper we shall show that the contents of the parietal cells and of the lumina of the gastric glands are not acid but neutral or alkaline, even when hydrochloric acid is being actively formed on the surface of the mucous membrane.

FACTS WHICH HAVE SUGGESTED SOME ASSOCIATION BETWEEN THE PARIETAL CELLS AND THE HYDROCHLORIC ACID OR ITS ANTECEDENTS.

Heidenhain ('70), Langley ('81) and others were able to associate other secretory functions of the gastric mucous membrane very definitely with other cells. This left the parietal cells and the formation of hydrochloric acid. Further, Miss Greenwood ('85), and afterward Macallum ('08) and Miss Fitzgerald ('10), have shown that chlorides are more abundant in the parietal cells than in other parts of the gland. These facts suggest that there is some association between the formation of the hydrochloric acid and the parietal cells. They leave the question quite open, however, as to where the free hydrochloric acid is first formed as such. The parietal cells probably form substances which later furnish the chlorine of the hydrochloric acid, but our results appearing in the second part of this paper show that these cells do not normally contain the hydrochloric acid itself.

Miss Fitzgerald ('10) employed the Prussian blue reaction and obtained very definite results which must be carefully considered in the decision of this question. She found the Prussian blue deposited in the canaliculi of some parietal cells. It will be worth while to examine this reaction and the varying results which have been obtained with it.

The Prussian Blue Reaction.

This was first employed by Claude Bernard ('59) in his classic experiment. A translation of his account is as follows: "In a rabbit which had eaten very little there was injected into the jugular vein a solution of lactate of iron and then a solution of prussiate of potassium; both solutions were warm. Three quarters of an hour afterward the animal was killed and at the autopsy it was impossible to demonstrate the blue color in the tissue of any organ. The urine, which was alkaline and cloudy, was not blue, although it contained both prussiate of potassium and the iron lactate, for it sufficed to add a few drops of hydrochloric or sulphuric acid to cause the blue color of Prussian blue to appear immediately. Upon opening the alimentary canal a blue color was found on the surface of the mucous membrane of the stomach and particularly on the part which corresponded to the lesser curvature of that organ. But this blue was quite superficial; the little deposits of Prussian blue were only on the surface of the mucous membrane, and a microscopic examination did not reveal any Prussian blue in the gastric glands."

Later, Claude Bernard ('77) said: "The acid of the gastric juice is formed only after the secretion of the juice, the glands secreting a liquid which breaks up into an acid fluid and another product as yet not definitely determined."

The results which we have to report indicate that the decision reached by the great French physiologist is correct.

The Prussian blue reaction was employed also by Lepine ('72) in dogs. He used potassium ferrocyanide with lactate of iron or sulphate of iron. He was unable to obtain Prussian blue in any cells of the gastric gland either by injection, maceration, or by passing the salts through a dialyzing membrane made of the gastric mucous membrane, although by the latter method he did obtain a little blue in a lymphatic space of the connective tissue between the glands. He concluded that the acid was not formed as such within the gland.

Sehrwald ('89) put pieces of the gastric mucous membrane into a solution of lactate of iron for one day and later into a solution of potassium ferricyanide. He believed that in this way he would obtain a deposit of Prussian blue at the seat of

formation of the acid. His results were not definite, although he thought the parietal cells showed more blue than other parts of the mucous membrane. The experiment was repeated by Miss Fitzgerald, who found the results too indefinite to decide the question, and by ourselves with the same outcome.

Fitzgerald's Paper.

Miss Fitzgerald ('10) conducted very careful and elaborate experiments by this method and has obtained by far the most definite results. Her experiments and the results are set forth in the following table, which is copied from her paper:[1]

We would like to direct especial attention to a few facts reported n this table and in her paper. First, the plates accompanying her paper show very clearly the deposit of Prussian blue within the canaliculi and in other parts of some parietal cells and also in the interglandular blood and lymph vessels, and in wandering cells and leucocytes. Second, in some experiments the Prussian blue reaction was not obtained. Third, when it did appear it was obtained in only one part of the stomach, namely, that near the œsophagus and along the lesser curvature. This is not the part which contains parietal cells in greatest abundance. Fourth, even in this region only a few of the parietal cells showed the Prussian blue. Those of the deeper third of the gland tubules, that is, the third farthest from the free surface never contained it; and in that part of the gland tubule where it did appear it was found in only a fraction of the parietal cells, so that altogether she found it in only a small percentage of the total number of parietal cells of the stomach, and this notwithstanding the fact that during some part of the long time the experiments lasted all parts of the mucous membrane must have been in full digestive activity. Fifth, it appeared in other structures as well as in the parietal cells, namely, in the blood vessels, in the connective tissue spaces and lymphatic vessels, in wandering cells, and in leucocytes. Sixth, in two instances (rabbits 5 and 6) it did not appear in the canaliculi of the parietal cells but only at the surface of the latter remote from the lumen and next to the blood vessels.

Our own results have confirmed these facts entirely.

TABLE GIVING RESULTS OBTAINED BY INJECTING A SOLUTION CONTAINING EQUAL PARTS OF AN AQUEOUS SOLUTION OF 2.25 PER CENT. AMMONIUM FERRIC CITRATE AND OF 1.5 PER CENT. POTASSIUM FERROCYANIDE.[1]

Animal.	Mode and No. of Injections.	Amount Injected. C.c.	No. of Hours Since First and Last Injections.		Condition.	Toxic Effect.	Prussian Blue Reaction—Spontaneous.			Ditto on Addition of 0.5 Per Cent. HCl.	
							Gastric Mucosa.		Skin.	Urine.	Various Tissues.[2]
			First.	Last.			Surface.	Interior.			
Rabbit (1) . . .	Intravenous, 1 Subcutaneous, 2	10–11	35¼	2	Food given.	−	−	−	−	+	+
Rabbit (2) . . .	Subcutaneous, 8	37	30	2½	Food given.	−	+	+	+	+ (1st day) − (2d day)	+
Rabbit (3) . . .	Subcutaneous, 5	31–32	25¾	2½	Starved 24 hrs.	− (1st day) + (2d day)	+	−	−	− (Retention of urine)	+
Rabbit (4) . . .	Intravenous, 2	17	4½	2¾	Semi-fasting. (Starved 36 hrs. Food. Starved 24 hrs.)	−	−	−	−	+	+
Rabbit (5) . . .	Subcutaneous, 5	50	6½	1	No food eaten during expt.	+	+	+	−	+	+
Rabbit (6) . . .	Subcutaneous, 4	43–45	3	¾–1	Food before exp.	−	+	+	−	+	+
Rabbit (7) (sod. ferrocyanide)	Subcutaneous, 4	40	5¼	1	Semi-fasting. Food given after fast of 20 hrs. and during expt. Eaten well until after 4th injection.	+	−	−	−	+	+
Guinea-pig (1)	Subcutaneous, 2	22	3¾	1¾	Food given.	+	+	−	+	+	+
Guinea-pig (2)	Subcutaneous, 2	16	5¼	3	....	+ (Polyuria)	+	−	−	+−?	+
Guinea-pig (3)	Subcutaneous, 2	19	6¾	4½	....	+ (Polyuria)	+	−	+	+	+
Dog (1)	Subcutaneous, 2	55	3¼	1¼	Starved 48 hrs. Food given 1 hr. before expt.	+ (Vomiting)	−	−	−	−	+
Dog (2)	Subcutaneous, 2	42	3¼	..	....	+ (Comatose)	+	−	+	+	+

\+ means positive result. − means negative result.

[1] M. P. Fitzgerald, 1910, *Proceed. Roy. Soc.*, B. 83.

[2] After their removal from the body, and subsequent exposure to the action of dilute hydrochloric acid, the Prussian blue reaction developed, at various times, in the following tissues: Bladder, kidney, liver, small and large intestines, ovary, fallopian tubes, uterus, vagina, and lymph gland of axillary region (dog). The kidney became quite blue in many cases, and under the microscope the uriniferous tubules presented a very striking appearance.

From these results Miss Fitzgerald concludes that the hydrochloric acid was formed as such in the parietal cells and secreted into the gland lumina. She says (p. 82): "The occurrence of the Prussian blue reaction in the canaliculi of the parietal cells of an animal injected with a solution of these two salts affords conclusive evidence of the presence of free acid within these structures."

The absence of the Prussian blue from most of the parietal cells might be regarded as evidence that only a few of these cells are engaged in the formation of hydrochloric acid, and that the greater part of them do not form it. She explains this absence from most of the parietal cells by saying that it may have been washed out of them, but surely in that case it would have appeared in them in some preparations. She explains the appearance of the reaction in the blood vessels and in the part of the parietal cell next to the blood vessel (and that part only) by the suggestion that under certain circumstances the acid may be secreted by the parietal cells into the blood stream instead of into the gland lumen. But the facts she reports are open to the other interpretation that the Prussian blue, or the salts forming it, may have been excreted from the blood stream into the parietal cell.

Our Own Experiments.

These were conducted upon rabbits, cats, dogs, a fowl, a snapping turtle, and several skates. Into these animals we injected solutions of sodium ferrocyanide (which we found less toxic than potassium ferrocyanide) and solutions of iron and ammonium citrate. We did not always use molecularly balanced solutions, because we found that the two salts were excreted with very different degrees of rapidity and by different ways. The injections were made subcutaneously or intravenously. In so far as our experiments repeated those of Miss Fitzgerald, they confirmed the results reported by her entirely, but we obtained also some additional results which have a very important bearing upon the conclusion which she drew.

By these experiments we sought to get answers to the following questions: First, is the Prussian blue precipitate produced in any place other than the gastric mucous membrane?

Prussian Blue in Places Other than Gastric Mucous Membrane.—Into the ear vein of a rabbit was injected 13 c.c. of a 10 per cent. solution of sodium ferrocyanide, and into the other ear vein 10 c.c. of a 25 per cent. solution of iron and ammonium citrate; both solutions were warm. They were perfectly fresh, having just been made. They did not give a precipitate of Prussian blue when diluted each with an equal quantity of water, one added to the other, and the mixture allowed to stand in a warm chamber at 37° C. for 28 hours. The rabbit showed some toxic effects during the injection, rallied temporarily, but died in 20 minutes. The stomach was opened at once. It was full of fresh food in an active state of digestion. Prussian blue appeared in all parts of it, although the lesser curvature seemed to have less of it than the rest of the stomach. Pieces of various tissues were fixed in absolute alcohol. The urine showed both salts present in abundance; the bile contained none of either. The saliva contained ferric citrate but no ferrocyanide. The heart's blood showed no blue on the addition of acid nor on the addition of acid with each one of the two salts. Therefore, it did not contain either salt in any quantity appreciable by this method. Paraffine sections were cut and they showed the Prussian blue reaction on the surface of the stomach; in the mucous membrane of the stomach appearing in the blood vessels, in the lymphatic spaces, in the epithelium between the foveolæ; in the connective tissue of the muscularis mucosæ and of the tunica muscularis of the stomach. It did not appear in the parietal cells nor in any other gland cells, nor in the gland lumen. It was found in many other tissues, namely: in the liver, appearing in the blood vessels, in the endothelial cells of Kupffer, and a little in the bile capillaries; in the spleen appearing in the blood vessels; in the blood vessels of the heart muscle. These were the only places in which we looked for it, but we found it in all of them.

This experiment was repeated, using smaller quantities of the salts injected subcutaneously on two successive days (a total of 1.6 grammes iron and ammonium citrate and 3.0 grammes sodium ferrocyanide well diluted). The rabbit was killed 30 minutes after the second injection; the tissues were fixed in formalin (neutralized with magnesium carbonate). The Prussian blue

reaction was found in all places in which it was found in the first rabbit. In addition it was found in the villi of the duodenum appearing in the connective tissue near the free end of the villus, and also between the epithelial cells of the free end of the villus. The blood vessels of the gastric mucosa contained Prussian blue, but in much smaller quantities than did those of the spleen and liver. The parietal cells were practically free of it; there may have been a little, for three or four minute particles were found in the section, but it was impossible to say that these extremely minute particles were not on the surface of the cell instead of within it.

From these two experiments it is evident that the Prussian blue is precipitated in the blood stream when solutions of these salts are injected into it. It may also be precipitated or absorbed in various places, especially in the endothelial cells of Kupffer in the liver. They show that it may appear on the surface of the stomach when it is not in the parietal cells; that it appears in the blood vessels and lymphatic spaces before it appears in the parietal cells. Its appearance between the cells of the surface epithelium between adjacent foveolæ, and also in the interglandular blood vessels and lymphatic vessels beneath this epithelium suggests that the Prussian blue or some or all of the salts necessary for its formation may pass from the surface into the interglandular lymphatics. These results taken in conjunction with those reported by Miss Fitzgerald in her rabbits Nos. 5 and 6 suggest very strongly that the Prussian blue or the constituents forming it may pass from the blood vessels into the parietal cells instead of in the reverse direction as she supposed. In other rabbits we have frequently seen the Prussian blue in the blood vessels and in only those parts of a few parietal cells lying next to the blood vessels, just as she reports in her rabbits 5 and 6. We have never found it in the parietal cells without finding it also in the blood vessels, but in these two instances we found it in the blood vessels when it was absent from the parietal cells. When it was present on the surface of the stomach we found it uniformly present also between the cells of the interfoveolar surface epithelium and in the interglandular lymphatic vessels and blood vessels. We have fre-

quently found it similarly in the tips of pyloric and duodenal villi, between the epithelial cells and in the subjacent connective tissue. Similar results were obtained in a fowl which had been given subcutaneously 13 c.c. of a 10 per cent. solution of sodium ferryocyanide and 13 c.c. of a 25 per cent. solution of iron and ammonium citrate, and two hours later intravenously 7 c.c. of the sodium ferrocyanide solution and 4.5 c.c. of the iron and ammonium citrate solution; both solutions were fresh and warm. The animal stood these injections very well, although they had some toxic effect. The fœcal passages were liquid and contained a little blue precipitate which became quite heavy and abundant on the addition of hydrochloric acid. The animal was killed in one hour by chloroform, and pieces of various tissues were fixed in absolute alcohol. When the proventriculus was opened a blue fluid welled out of the openings of the depressions which contain the compound glands; no blue color appeared elsewhere in this organ. Microscopic examination of paraffine sections showed Prussian blue on the surface of the epithelium lining the necks of the depressions of the surface epithelium into which the glands open and in the lumina of these depressions. It was not within the glands themselves. It was present between the epithelial cells of the region where the depression receiving the secretion of the glands opened on to the surface of the proventriculus and in the subjacent blood vessels and lymphatic spaces. It was abundant in the blood vessels and lymphatic spaces of the muscular coat of the stomach, and of the crop, and of the heart muscle, and of the muscular stomach (gizzard). It appeared also in the epithelium of the intestine and in the connective tissue and muscle of the cœcal diverticula. It was abundant in the liver appearing in the endothelial cells of Kupffer and in the blood vessels. It was absent from the breast muscle and its lymphatic and blood vessels.

These results, therefore, answer this first question in the affirmative. The Prussian blue reaction appears in many places besides the gastric mucous membrane. In most of them it seems probable that it takes place without the help of an acid. And they show that, if its occurrence on the surface of the gastric mucous membrane is due to the acid formed in the stomach then

that acid is present on the surface while it is absent from parietal cells and from the gland lumen.

Effects of Injury on Amount of Prussian Blue Precipitate.—Second, is the amount of Prussian blue precipitate increased by mechanical injury of the mucous membrane? A small kitten was given by subcutaneous injection small doses of a mixture of solutions of potassium ferrocyanide and iron and ammonium citrate in molecular proportions. These injections were given three times a day for three days. On the third day the abdomen was opened, a small piece of mucous membrane was removed from the fundus region of the stomach, the wound in the mucous membrane was sutured with silk sutures and afterward the other coats of the stomach were united and the abdomen was closed. The operation was done with aseptic precautions. Five hours later the kitten was killed, pieces of tissue were fixed in alcohol and in neutral formalin (freshly distilled over potassium hydrate). In the immediate vicinity of the suture paraffine sections showed that the Prussion blue reaction occurred in great abundance in the blood vessels, in the lymphatic spaces and in the parietal cells. These preparations showed blue in a very large proportion of the parietal cells and even in those at the bottom of the tubules. It was especially abundant in the parietal cells of the necks of the glands. Many parietal cells were dead and thrown off into the gland lumen; in every instance these dead cells were filled with Prussian blue. In those cells which were still in their normal position many showed the Prussian blue in the canaliculi disposed in a manner very similar to that which Miss Fitzgerald has shown in her Plate VII., Figs. 6 and 8. As one proceeded in the study of these sections progressively farther from the site of injury the amount of Prussian blue and the number of parietal cells showing it progressively decreased, and in some sections of mucous membrane taken from parts of the stomach remote from the site of operation and apparently in a healthy condition, the parietal cells did not contain any blue at all.

In another cat a similar operation was performed on the gastric mucous membrane and during four successive days following solutions of potassium ferrocyanide and of iron and ammonium

citrate in equal quantities were injected subcutaneously. On the fourth day the cat died, but the tissues were immediately fixed in neutral formalin. There was more Prussian blue in the immediate neighborhood of the sutures than in other parts of the mucous membrane.

These results answer the second question in the affirmative. And they show that dead parietal cells show more Prussian blue than living ones. The dead ones are always stained by it, whereas most of the living ones are not. This suggests that the death of the parietal cell or a lowering of its vitality may permit or facilitate the penetration of the cell by substances concerned in the Prussian blue reaction, and that dead cells become acid.

Effect of Poisons on Amount of Prussian Blue Precipitate.—Third, is the extent of the Prussian blue reaction increased by the injection of poisons into the wall of the stomach? We injected into the submucous tissue of the stomach exposed under aseptic precautions, solutions of phosphorus in olive oil, and solutions of moccasin venom in distilled water. The incisions in the abdominal wall were closed and salts of sodium ferrocyanide and iron and ammonium citrate injected subcutaneously for varying periods. The results were negative; we could not demonstrate any increase of the Prussian blue reaction in the area where these poisons were injected.

Effect of Restriction of Blood Supply on Amount of Prussian Blue Precipitate.—Fourth, is the amount of Prussian blue increased in areas of restricted blood supply?

Arteries and veins of various sizes were tied on the stomachs of different rabbits and cats. Solutions of sodium ferrocyanide and iron and ammonium citrate were injected subcutaneously during several days. The results answered this question in the negative. There was no increase of Prussian blue in the areas of restricted blood supply. In a few cases ulcers were produced and on the surface of these there was always a considerable deposit of Prussian blue. The cells on the surface of the ulcer belonging to various parts of the gastric glands and presumably dead or dying always showed a considerable deposit of Prussian blue in them.

Precipitates Do Not Back into Gland Lumina from the Surface.—

Fifth, is the occasional presence of Prussian blue in the gland lumen and in the canaliculi of the parietal cells due to backing up of the blue precipitate from the foveolæ or from the surface of the mucous membrane.?

In order to determine this question many experiments were made with Prussian blue, with carmine and with India ink. Hydrostatic pressure, dialysis, and positive pressure by a syringe piston against a piece of gastric mucous membrane tied over the end of the syringe were employed. The results were all negative, thus confirming those of Lepine ('72).

Prussian Blue Reaction in Animals which Have no Parietal Cells, but Secrete Acid.—Sixth, where does the Prussian blue reaction occur in animals which have no parietal cells but yet secrete acid?

The experiment above reported with the fowl showed that the Prussian blue occurred in the lumen of the depression receiving the secretion of the compound glands of the proventriculus. It was not found in the gland cells. It appeared between the cells of the surface epithelium immediately adjacent to the opening of the depressions. As above stated, it occurred also in many other places.

A snapping turtle was given one half gram each of sodium ferrocyanide and iron and ammonium citrate in dilute solution three times daily during four days; the solutions were given separately and subcutaneously in the inguinal region. Two small fish were shoved into the stomach with a glass rod and were found there later partially digested. On the fourth day the animal was killed and the stomach examined. A deposit of Prussian blue was found in every cell of the somewhat coarse foveolæ of the gastric gland. In these cells it occupied a definite position, the same in each cell. Each cell contained a mucous plug which occupied the half of the cell next the lumen, under it a small spherical mass of Prussian blue, and under that the nucleus. The mass of Prussian blue was nearly as large as the nucleus. Sections through these foveolæ stained with paracarmine or mucicarmine presented a very pretty appearance because of the extreme regularity of the position of the nuclei, the little masses of Prussian blue and the mucous plugs. There

was more Prussian blue in the cells of the foveolar epithelium then anywhere alse. The amount in the cells of the necks of the gland was less and decreased toward the bottom of the gland tubule, although it appeared in nearly all the cells of the gland. It was abundant in the interglandular connective tissue, being in the blood and lymph vessels.

Skates experimented upon in the same way showed Prussian blue in the blood vessels and lymphatics of the wall of the pharynx and stomach. The glands of the gastric mucous membrane showed the reaction in small quantities in the gland lumina and in a few of the cells. The cells showing the reaction were more numerous in the part of the gland near the free surface than in its deeper portions. The surface epithelium of the stomach and of the intestines two inches beyond the pylorus and of the large intestine contained small quantities of Prussian blue.

Summary of the Results Obtained by the Prussian Blue Reaction and their Significance.

From the facts reported by Miss Fitzgerald and those which our own experiments have added, it seems clear that the occurrence of the Prussian blue reaction does not necessarily indicate the formation of free mineral acid under normal conditions in the places in which it is found. It appears in many places where it could hardly have been due to the presence of acid—in the blood, lymph, liver, spleen, intestine, heart muscle, etc. In these places its presence must be due to something else.

It may be due to fatty acids, which bring about the precipitation of Prussian blue from solutions of the salts used in these experiments in proportion to the amount of the acid present. It may be due to the withdrawal of the ammonium citrate by more rapid diffusion or by the involvement of the ammonium in the metabolic processes of the tissues. Nencki and Pawlow ('96) have shown that the gastric mucous membrane normally contains an extraordinarily large amount of ammonia. Ferric citrate and sodium ferrocyanide solutions give when mixed an immediate precipitate of Prussian blue even in the absence of any acid. It may be due to the death or reduced vitality of cells, permitting

the entrance of salts which would not have entered living or healthy cells. It may be that the cells which show the reaction are just those which are poisoned by the salts used. The iron and ammonium citrate solution injected repeatedly during several days caused the death of one kitten. Therefore this salt has a serious toxic action. It may be that the interaction chemically of these two salts with the contents of certain cells may sometimes permit the liberation of an acid in them, when no acid would have been produced in the absence of the complex chemical state which exists when they are present. It may have been absorbed from the surface of the mucous membrane. Its presence between the epithelial cells and in underlying lymphatic vessels of the stomach and intestine suggest this possibility.

Since the Prussian blue may be precipitated in so many places, the fact that it is sometimes precipitated in the canaliculi of a few parietal cells in a relatively small part of the stomach perhaps in an abnormal condition at the time does not necessarily prove that free hydrochloric acid is formed under normal conditions in the parietal cells of the stomach as a whole. The failure to get any reaction in the stomach in some experiments, the small number of parietal cells in which it ever appears, its occurrence in other tissues and in the blood vessels and lymph vessels before it appears in the parietal cells at all, the fact that several factors other than the presence of free mineral acid may cause the precipitate to form,—all these things show that it would not be right to conclude from the evidence which the Prussian blue reaction affords that free hydrochloric acid is formed in the parietal cells. Much less could one reach this conclusion from any other evidence that has been adduced, for all other evidence is much less definite than this. And this failure to show clearly that free hydrochloric acid is formed in the parietal cells becomes quite clear when it appears, as we shall show in the following part of this paper, that the contents of the canaliculi of these cells are alkaline and those of the gland lumina are not acid when free acid is being produced by the mucous membrane.

EXPERIMENTS WITH INDICATORS: THE REACTION OF THE SECRETION WITHIN THE LUMEN OF THE ACTIVE FUNDUS GLAND, AND WITHIN THE INTRACELLULAR CANALICULI OF THE PARIETAL CELLS.

It is obvious from the observations of Fitzgerald and ourselves reported in the preceding section that the Prussian blue reaction is not a trustworthy indication of the place of formation of the hydrochloric acid of the gastric juice, and that we must look to other methods for a solution of this problem. One naturally turns to the chemical indicators for this purpose. The results obtained by means of these substances by previous investigators who have employed them have been uniformly unsatisfactory and unconvincing. The most definite results obtained by these methods are those reported by Fränkel ('91) who used neutral sodium rosanilinsulphonate as an indicator, and by Edinger ('79), who employed a solution of sodium alizarin.

Rosanilin sulphonic acid possesses the property of forming with sodium hydroxide acid salts which form red solutions (acid fuchsin), and neutral salts whose solutions are colorless. The addition of small quantities of acid to solutions of the neutral salt results in the production of the red colored acid salt. Accordingly, Fränkel injected into the jugular veins of dogs 50–100 c.c. of a 5 per cent. solution of the neutral sodium rosanilin sulphonate. As a result of this proceeding he found the entire mucous membrane of the stomach, including the pyloric mucous membrane, stained brilliant red. Teasing portions of the mucous membrane in distilled water he found that both parietal and chief cells were stained in the fundus glands, and that the cells of the pyloric glands were also stained, while the cylindrical cells of the surface were unstained. He could see no difference in the intensity of the stain in the two types of cells. The same experiment was also performed on rabbits, but in this case he found that the color was not uniformly distributed throughout the mucous membrane, and the pyloric mucosa showed only a few slightly red spots.

From these experiments Fränkel concludes: that the mucous membrane of the stomach has an acid reaction; that the acid is formed in the parenchyma cells; and that it can always be

demonstrated in them. Regarding the acid reaction obtained by this method in the pyloric region he does not venture an interpretation.

The results obtained by Edinger by means of the sodium alizarin reaction were similar to those of Fränkel. Sodium alizarin, as Edinger pointed out is, in neutral solutions, of a deep purple red color, while the addition of an acid results in the precipitation of the alizarin as a flocculent yellow precipitate. Edinger prepared the solution by adding alizarin in excess to a 10 per cent. solution of sodium hydroxide. Then the solution was filtered. 25–100 c.c. of this solution were injected into the jugular veins of rabbits and dogs. In a rabbit he found after this injection the stomach spotted red-violet and yellow, the latter being more general in the region of the greater curvature, though the pyloric mucous membrane was also yellow. He concludes that the glands of the rabbit's stomach are not all in activity at the same time, and that both the fundus and pyloric mucous membranes react acid. In dogs, after similar treatment, the whole mucous membrane of the stomach, including that of the pyloric region, was yellow. Sections of the mucous membrane showed that the yellow color was to be found at all levels, but the intensity of the stain was too slight to permit of the recognition of the stain in particular cells. The pancreas also gave an acid reaction.

Experiments with tropeolin, congo red, litmus, phenolphthalein, and other indicators in common use, have been without result or, at the most, have only indicated what was known from examination of the secretion, namely, that the contents of the stomach were acid.

It is obvious from the foregoing statements that the results of the experiments of Edinger and Fränkel with sodium alizarin and sodium rosanilin sulphonate were not in accord with what had been previously determined concerning the place of formation of hydrochloric acid in the stomach, inasmuch as they indicated the formation of acid in the pyloric mucous membrane which had previously been shown by Heidenhain ('70) and Klemensiewicz ('75) to secrete an alkaline fluid. Moreover, neither of these experiments gave any clear indication of the ource of the hydrochloric acid.

It is also clear that in order to solve this problem by the use of a chemical indicator the substance employed, in addition to being an indicator of acidity or alkalinity, must have the properties of a vital stain, that is to say, the cells of the gastric glands must be freely permeable to it and it must have a special affinity for constituents of the gastric secretion in the glands, or the distribution coefficient must favor its concentration in this secretion in sufficient amounts to give a distinct color reaction. These conditions we have found to be fulfilled by neutral red and by a number of dyes belonging to the naphtol blue series including nile blue, and the various cyanamins discovered by Witt ('90).

Our first successful experiments in staining specifically the secretion in the parietal cells and in the lumina of the gastric glands were obtained with Grübler's napthalin blue R crystals (a trade name for naphtol blue). Solutions of this dye in normal salt solution, injected into the blood vessels of the recently killed animal, were found to stain the secretion in the canaliculi of the parietal cells and in the lumina of the gland tubules of the fundus region a distinct red color, while the cells of the foveola and the mucus on the free surface were stained a deep blue.

Tests of the solution of naphtalin blue afforded no explanation of this result, inasmuch as addition of acid produced no change in the color of the solution, and addition of sodium hydrate gave a green color. Accordingly, it seemed probable that the reaction observed was due either to another dye present in the naphtalin blue as an impurity, or to a new dye synthesized during the process of staining.

After consideration of the commercial process for the manufacture of the naphtol blues it seemed probable, in view of the fact that dimethylparaphenylendiamin is a biproduct of the synthesis of naphtol blue from nitrosodimethylanilin and B naphtol, that the dye on which this reaction depended would prove to be cyanamin, which, according to Witt, is formed when the mixture of naphtol blue and dimethylparaphenylendiamin resulting from the synthesis above mentioned is boiled for a time with an alcoholic solution of potassium hydroxide. Accordingly, cyanamin chloride was prepared by the process described by Witt, and its solutions tested on the gastric mucous membrane.

Cyanamin, according to Witt, possesses two basic groups, one molecule of the base combining with two molecules of hydrochloric acid to form a bichloride which is soluble in water with a deep blue color. On dilution of this solution the compound is broken up into a monochloride insoluble in water, which deposits as a reddish violet precipitate, and hydrochloric acid, which remains in solution. On the addition of alkalies the solution changes to a red color and after a short time the base settles out as a red flocculent precipitate.

On account of the formation of a monochloride intermediate in color between the red base and the blue bichloride it is apparent that as an indicator of reaction cyanamin does not approach in delicacy of response the more commonly used chemical indicators. But when we consider the relatively high content of hydrochloric acid in the gastric juice (as high as .5822 per cent. according to Rosemann ('07)) this is of little importance, for we have found that in dilute solutions of the dye a concentration of .0009 per cent. of hydrochloric acid, or approximately 1/600 of the concentration in the gastric juice, is sufficient to abolish all trace of red color. Furthermore, if Pawlow's idea is true that the native secretion has a constant acidity, and that the variations in acidity of the secretion from a gastric fistula are due to different degrees of neutralisation by the alkaline mucous secretion of the surface epithelium, then we might expect a maximum acidity in the gland lumen assuming that the hydrochloric acid is secreted as such by the cells. It follows therefore that if cyanamin stains the gastric secretion in the glands it will stain it blue wherever the acid is produced.

The method of applying the cyanamin is as follows: A fresh concentrated solution of the bichloride in normal sodium chloride solution is prepared; the animal is killed by a blow on the head, or by bleeding from the carotid, and the stomach exposed as rapidly as possible; a small piece of the mucous membrane is cut out with scissors, rinsed in normal salt solution, and placed in the solution of the dye. A few minutes' immersion suffices to accomplish the staining. When this is complete the piece of mucous membrane is placed on a slide with the mucous surface downwards and observed with a low power of the microscope.

If the staining has progressed far enough the edge of the preparation may be teased with needles and the superficial glands which alone are stained so isolated, when a cover glass is applied and the preparation studied by high power objectives. For these experiments we have used rabbits, guinea pigs, cats, and dogs.

In such preparations certain cells scattered throughout the glands promptly stain blue, the blue color affecting not only the protoplasm but the nucleus. These belong to both classes of cells constituting the glands and are interpreted by us as dead cells. In addition the small cells, first described by R. Heidenhain, which occur in small numbers scattered among the other epithelial cells of the gland, and the nature of which is still obscure, stain blue, but in this case the blue stain is confined to the granules with which the protoplasm of these cells is studded, the nucleus remaining unstained. Certain glands on the very edge of the preparation may stain bluish red, these being for the most part glands which have been actually injured in making the preparations.

In the uninjured glands reached by the dye, on the contrary, a uniform and characteristic reaction is obtained. With the exception of the dead cells and the small cells of Heidenhain mentioned above, the dye is entirely confined to the secretion in the lumina of the glands and their various diverticula, including the whole basketwork of canaliculi in the parietal cells—all of which was intensely stained. Moreover, in no place in this system of gland tubules below the level of the gastric foveolæ was the blue color of the acid solutions of the dye obtained. On the contrary the secretion contained in the canaliculi of the parietal cells was a distinct red like that displayed by the dye in alkaline solutions, while the secretion in the lumen of the gland was a bluish red. The short canaliculi connecting the parietal cell system of intracellular channels with the main lumen of the gland showed a color shading from the red of the content of the latter to the bluish red of the contents of the gland lumen. At the level of the bottoms of the foveolæ the color of the secretion changed rapidly to the pure blue of the acid solutions of cyanamin, and the cylindrical cells of the surface and of the foveolæ stained the acid color also.

Inasmuch as the results just described indicated that in no part of the gland system below the foveolar level did the secretion of the gastric gland cells have an acid reaction, and that the secretory contents of the parietal cells were even alkaline in reaction it was important to test the behavior in different states of physiological activity of these glands towards solutions of the dye. These experiments were performed on dogs, animals being kept without food for twenty-four hours, and compared, as regards the reaction with cyanamin chloride, with other animals at different intervals after feeding. These experiments showed that the resting gastric glands gave no reaction with cyanamin, while glands taken from active stomachs fifteen or more minutes after secretion gave the pronounced and characteristic reaction described above. Accordingly, the alkaline reaction of the contents of the canaliculi of the parietal cells, and the non-acid reaction of the contents of the lumen of the gland proper, are not the reactions of resting glands, but only of active glands from a stomach which is forming an acid secretion.

The amount of cyanamin chloride at our disposal did not permit our testing its action on the stomach when injected into the living animal intravenously, or by injection immediately after death of solutions through the blood vessels. Naphtol blue, however, apparently owes its properties in this connection to admixture of cyanamin, or to synthesis of the latter during the process of staining, for the reaction which it gives is exactly that of the pure cyanamin solutions, and we have been able to separate from the commercial zinc naphtol blue double chloride small quantities of cyanamin. Naphtol blue dissolved in normal salt solution injected from the aorta in a rabbit killed shortly after feeding will produce this reaction in every gland of the fundus region of the stomach. It is difficult, however, by this method, to secure a staining of the entire gland, for reasons which a consideration of the blood supply of the mucous membrane will make apparent. The bases of the glands stain well, but it is difficult to secure a staining of the upper portions of the glands. Preparations made in this way give, however, the most remarkable demonstration of the canalicular system of the glands that we have ever seen, resembling except for the color a perfect silver chromate impreg-

nation of this system. We have also obtained a feeble reaction by means of intravenous injection of solutions of naphtol blue.

Confirmatory Tests.

In view of the results obtained with cyanamin chloride it seemed probable that other dyes closely related to this substance would give similar reactions, and accordingly we prepared by acting on naphtol blue with anilin, according to the method described by Nietzki and Bossi ('92), the closely related dye named by the former phenylated nile blue. Solutions of this dye gave by far the most striking results obtained inasmuch as the intensely red base was precipitated in the canaliculi of the parietal cell, while the secretion in the gland lumen was stained a bluish red color. Similar results were obtained with solutions of nile blue sulphate, but a less pronounced reaction was obtained, the content of the parietal cells staining in this case bluish red, that of the lumina of the glands blue.

Neutral red, which has been highly commended by Ehrlich as an indicator for biological studies, next suggested itself in this connection for we had long known that it stained the secretion in the gastric glands and in the parietal cells. This dye may be used like cyanamin by immersing the fresh mucous membrane in a 1 in 10,000 solution in normal salt solution or by injecting such a solution of the dye through the blood vessels. In neutral solutions neutral red possesses a reddish color with a suggestion of orange. Alkaline solutions precipitate the base in the form of a yellow precipitate while acid solutions produce a crimson color. This dye therefore is capable of indicating either acidity, alkalinity or neutrality. In preparations made as indicated above of the fresh actively secreting fundus mucous membrane of the stomach neutral red promptly stains the secretion in the canaliculi of the parietal cells and in the main lumen of the gland. In the parietal cells the color assumed is the unmistakable yellow of the free base, in the lumen of the gland the color approaches more closely to the neutral tint, while the short diverticula of the lumina which connect the parietal cell with the lumen are of an intermediate tint. The whole system, however, is without question on the alkaline side of the reaction with

neutral red. At the bottom of the foveolæ the alkaline reaction gives way to the crimson acid color which is exhibited by the whole foveola and by the surface. The foveolar epithelium also stains the crimson acid tint. Neutral red stains the dead cells deep red, and also the granules of the small cells of Heidenhain referred to above.

Thus the consistent results of four separate methods show that the hydrochloric acid is not free as such in the gland, and that the contents of the canaliculi of the parietal cell contrary to expectation are alkaline in reaction. The question naturally arises, then, where is the acid of the gastric juice formed and what are the factors concerned in its formation? Without doubt, our reactions with the dyes of the cyanamin series indicate that the hydrochloric acid of the gastric juice is set free in the foveola, possibly also on the free surface of the mucous membrane. As to the source of the chlorine concerned in the formation of hydrochloric acid of the stomach the experiments of Greenwood ('85), Macallum ('08), and Fitzgerald ('10) seem to be conclusive. Greenwood showed that in preparations of the mucous membrane of the stomach made with silver nitrate, and then reduced in the light, the parietal cells stained much more strongly with the silver deposit than the other epithelial elements. In his studies of the silver reaction for chlorides Macallum showed that only chlorides, phosphates, and carbonates, of silver gave this reduction reaction, and devised a method by means of which the phosphates and carbonates could be excluded and only chlorides exhibited. This method consisted in using for the reaction a solution of silver nitrate containing nitric acid in which the phosphates and carbonate of silver are soluble. By this means he demonstrated that the parietal cells of the stomach were rich in chlorides. This result has recently been confirmed by Miss Fitzgerald, who found that the reaction was obtained not only in the body of the parietal cell but also in the intracellular channels.

This being the case, in view of the fact that the secretion of the parietal cells is alkaline while in the cells themselves, and that the secretion of the whole gland while contained in the gland lumen is very nearly neutral as shown by the neutral red and cyanamin reactions, it seems probable that the chlorine is

secreted by the parietal cells in the form of a chloride of an organic base, and that the hydrochloric acid is only set free after this secretion is poured out of the gland into the foveola. As to the nature of this base, there are some facts which suggest the probability that it is protein in nature. Stöhr's ('82) description of the parietal cells in man indicates clearly that he perceived a coagulated substance in the canals which connect these cells with the lumen of the gland, and Revell has succeeded in staining the content of the intracellular canals of the parietal cells with carmin solutions in material fixed in an alcohol bichromate sublimate mixture.

THE CONSISTENCE OF THE SECRETION IN THE GLAND LUMEN.

The fact that we were able to stain the secretion of the gastric glands while still contained in the gland has enabled us to study certain properties of this secretion. In the actively secreting rabbit stomach the lumen of the gland is widened by the accumulation of the secretion, and, by pressure on the cover glass, or by teasing, it is possible to expel the secretion from the gland, or to liberate it in the salt solution used for mounting and thus to learn something about the change in concentration of the secretion which takes place as it proceeds towards the surface of the mucous membrane. The assumption which is generally made that the secretion is formed by the glands in the same concentration as it presents when it emerges from the openings of the foveolæ, would lead one to suppose that the secretion in the gland would be a limpid solution, which would flow easily from the gland, and would mix readily with salt solution. This, however, proved not to be the case. When water from the surrounding salt solution enters the gland lumen the column of secretion breaks up into round droplets which maintain their individuality for several minutes. Similarly, when secretion is expressed from the gland lumen into the surrounding solution it collects around the mouth of the gland in large spherical droplets which slowly dissolve, the red reaction also at the same time slowly changing to the blue acid reaction, if the secretion has been stained with cyanamin. From these observations we are obliged to conclude that the secretion formed in the gland possesses a

relatively high content of solids, and that the bulk of the water found in the gastric secretion is added at the level of the glandular foveolæ.

OTHER CONSIDERATIONS.

Since it is apparent that the contents of the gastric glands proper when in a state of normal activity are not acid in reaction, and may even be alkaline, it follows that the ferment of the same secretion in the gland lumen is probably not in an active form, since, as is well known, pepsin is destroyed by alkalies while pepsinogen is not affected. Hence, the failure of the secretion to attack the cells themselves requires no further explanation in the case of the gastric glands than in the case of the pancreas, since in neither case does the activated ferment come in immediate contact with the parenchyma cells. This being the case it is pertinent to enquire whether under any conditions the secretion within the gland may become acid in reaction, for, in this event it is probable that the ferment would be activated and as happens under similar conditions in the pancreas the adjacent parenchyma cells would be attacked. This possibility is suggested by certain results obtained with the Claude Bernard reaction, where a reaction was obtained in the neighborhood of recent injuries to the mucous membrane far down the lumen of the gland, though the rest of the mucous membrane showed no reaction in the glands. We have as yet not had the opportunity to test this question by means of the cyanamin and neutral red reactions, but hope to report on this matter in the near future.

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THE PHYLOGENY OF THE NEMOCERA, WITH NOTES ON THE LEG BRISTLES, HAIRS AND CERTAIN MOUTH GLANDS OF DIPTERA.[1]

W. WESCHÈ, F.R.M.S.

Professor Williston has published a paper[2] on the antennæ of Diptera which is not only a remarkable analysis of these organs, but one showing an encyclopædic knowledge. His deductions combined with his observations on other characters seem to me of great weight and importance, and may lead to a more exact classification of the order.

On page 326 of the cited paper, in commenting on the number of antennal joints in the different families of Diptera, he says: "We are at once struck with the predominance of five groups having a maximum normal number of sixteen, fifteen, ten, six and five. And I venture to suggest that these five groups represent in the main five different divergent phyla of Diptera." These groups are:

Group 1.—Families having from twelve to sixteen joints: Tipulidæ, Cecidomyidæ, Psychodidæ, Mycetophilidæ, Pachyneurinæ, Rhyphidæ.

Group 2.—Families with from six to fifteen joints: Dixidæ, Culicidæ, Blepharoceridæ, Chironomidæ.

Group 3.—Families with from seven to ten joints: Scatopsinæ, Simulidæ, Xylophaginæ, Stratiomyidæ, Acanthomeridæ, Tabanidæ.

Group 4.—Families with from three to six joints: Nemistrinidæ, Lonchopteridæ, Phoridæ, Cyclorrhapha.

[1] The MS. of the present article was sent to me some time before his death by the late Mr. Weschè, with a request for comment and criticisms. In editing the paper, which Mr. Weschè had not quite completed, I have made no changes whatever except verbal ones, and have omitted only a few immaterial parts. Most of his conclusions seem well taken, and it is to be regretted that the author could not have been spared to continue his researches along the fruitful lines that he had begun.—S. W. WILLISTON.

[2] BIOLOGICAL BULLETIN, XIII., p. 324, 1907.

Group 5.—Families with from three to five joints: Mydaidæ, Apioceridæ, Asilidæ, Therevidæ, Bombylidæ, Dolichopodidæ, Empidæ.

The Orphnephilidæ (11, 12), Bibionidæ (8–12), Leptinæ (3–8) and Scenopinidæ (3) fill in gaps between the chief groups. But it is obvious that the author was not dogmatic in the formulation of these groups, as later it is stated (p. 330) that "the antennæ, taken separately, are only partial evidences of relationship. They must be correlated with all other organs of the body, and must harmonize with theories based upon other organs." Carrying out this idea, and quite agreeing that no single character can be relied upon, I have endeavored to test the validity of these phyla by other structures, for the most part microscopic, such as the eyes, the trophi, and the genitalia; and I have embodied the results of my studies in a series of tables.

These tables have been made to show the dominant characters of the families; every large family has numerous exceptions and specializations, as, for instance, the labium in the Dolichopodidæ is nearly always short, though we know that it is long in *Orthochile*, and longer than normal in *Gymnopterus*. In speaking of dominant characters I must guard myself by saying that my cabinet of dissections is composed mostly of the commonest and most widely distributed forms, selected where the material for dissection was most abundant; there is, hence, probably a margin of error.

The tabulated observations are mostly contained in four papers previously published by myself: "The Mouthparts of the Nemocera," 1904, with additions and corrections (1909), (*Journal of the Royal Microscopical Society*); "The Genitalia of the Sexes in Diptera" (*Trans. Linn. Soc.*, London, 1906), and "The Structure of the Surface and the Sexual Characters of the Eyes of Diptera" (*Journal Queckett Club*, 1909). Many additional facts since observed have been incorporated in the tables.

The following explanations will be necessary for a full comprehension of these tables. The trophi are fully analyzed. Their most important parts, from the point of view of phylogeny, seem to be the tracheæ of the paraglossæ, the mentum, the palpi, and the pharyngeal pump. The tables of the genitalia give the

characters of the interior and more invisible parts, which I have formulated in a previous paper.[1] They will be described as of certain types:

Type 1.—A long flagelliform tube, as in *Tipula*, or an approximation to that type.

Type 2.—A prominent chitinous bulb, with lateral processes, as in *Ptychoptera*.

Type 3.—A low membranous process supported by chitinous levers, as in *Gymnoplista* and *Culex*.

In the second case the nature of the ancillary claspers are given (*a*) as simple hooks, as in many Muscidæ, (*b*) jointed, as in the Tabanidæ, (*c*) or a simple unsegmented cercus-like appearance, as in the Bibionidæ. Three types of ovipositor are differentiated:

Type 1.—Telescopic or protrusile, as in *Calliphora* or *Dolichopus*.

Type 2.—Nontelescopic, as in *Tipula*, where it can scarcely be said to exist.

Type 3.—Short segmented, such as is found in the Empidæ.

Further the appendages of the egg-guide are tabluated, as (*a*) cercus-like, as in *Bibio* or *Musca;* (*b*) uncinate, as in *Tipula* or many Muscidæ; (*c*) styliform, as in *Psychoda* or *Pipunculus*, where it is probably a fusion of the pair of forceps.

The number of receptacula is noted. The types of penis and ovipositor seem to me the more important characters. Among the characters of the eyes, holopticism, dichopticism, and the greater width of the female front are given; and the absence or presence of ocelli is also noted.

It may be stated that in certain families the compound eyes are chitinous plates pierced by circular facets or lenses; this opaque structure is much reduced in other families till only strips of chitin separate the hexagonal facets. Finally in specialized groups all traces of opaque structure are lost.

These tables may be examined in two ways. The most important characters may be noted and the tables consulted to see how far they are in agreement; or the characters of the group may be added up and averaged. The latter method shows that

[1] "Notes on the Value of the Genitalia in Phylogeny." *Trans. Entom. Soc. London*, 1908.

TABLE I.

MOUTH CHARACTERS OF THE NEMOCERA.

	Group.	Labium.				Tracheæ.		Mentum.				Labrum.				Hypopharynx.			Palpi.					Sense Organs.		Mandibles.				Maxillæ.			Pharyngeal Pump.			
	Williston's Phyla.	Short.	Long.	Membranous.	Chitinous.	Present.	Absent.	Undeveloped.	Developed.	Extremely Developed.	Median Suture.	Strong.	Weak.	Ciliated.	Vestigial.	Ciliated.	Weak.	Vestigial.	Maxillary.	Labial.	4 Joints.	2 Joints.	1 Joint.	Absent.	Present.	Peculiar.	Fully Developed.	Vestigial.	In Ventral Side of Labium.	Fused into Dorsal Side of Labium.	Laciniæ Present.	Stipites and Cardines Present.	Vestigial.	3 Plates.	2 Plates.	Vestigial.	Absent.
Cecidomyidæ	1	*					*	*				*				*			*					*						*	*					
Mycetophilidæ	1	*					*	*				*				*			*					*						*	*					
Bibionidæ: *Bibio*	0	*					*	*				*				*			*	*				*							*	*					*
Bibionidæ: *Dilophus*	0		*				*	*				*				*	*			*				*							*	*					*
Bibionidæ: *Scatopse*	3	*		*			*	*	?			*	*			*			*	*			*	*	*					*	*					*
Simulidæ	3	*		*			*	*				*				*			*					*												
Chironomidæ	2	*		*			*	?	*			*	*			*			*					*						*	*					
Ceratopogon	2		*	*			*	*	?			*	*			*			*					*	*		?[2]									
Psychodidæ	1	*		*			*	*	*			*	*			*			*					*						*	*					
Culicidæ: *Corethra*	2	*					*	*	*		?	*	*			*			*					*						*	*					
Culicidæ: *Mochlonyx*	2	*			?		*	*	*		?	*	*			*			*					*						*	*					
Culicidæ: *Culex*	2		*		*	*				*		*	*			?		*		*		?		*			♂				*	*					
Ptychoptera	1	*		*			*	?	*			*	*			*	*		*		*			*							*	*					
Tipulidæ	1	*		*		*		*				*	*			*			*		*			*							*	*					
Rhyphidæ	1	*		*			*	*			*	*		*		*[5]			*		*			*	*	*					*	*					

[1] Only a remnant of the fused parts.

[2] In one species.

[3] Fused together and in the same condition as the other Tipulidæ.

[4] This clearly shows as three in one preparation. I am not certain of two.

[5] Very vestigial cilia.

TABLE II.

CHARACTERS OF MOUTH IN BRACHYCERA AND CYCLORRAPHA.

	Group.	Labium.				Tracheæ.		Mentum.				Labrum.				Hypopharynx.				Palpi.				Sense Organs.			Mandibles.				Maxillæ.			Pharyngeal Pump.			
	Williston's Phyla.	Short.	Long.	Membranous.	Chitinous.	Present.	Absent.	Undeveloped.	Developed.	Extremely Developed.	Median Suture.	Strong.	Weak.	Ciliated.	Vestigial.	Ciliated.	Weak Unciliated.	Strong Unciliated.	Vestigial.	Maxillary.	Labial.	4 Joints.	2 Joints.	1 Joint.	Absent.	Present.	Peculiar.	Fully Developed.	Vestigial.	In Ventral Side of Labium.	Fused into Dorsal Side of Labium.	Laciniæ Present.	Stipites and Cardines Present.	Vestigial.	3 Plates.	2 Plates.	Vestigial.	Absent.
Asilidæ	5	*				*						*				*				*			*	*			*				*	*					*
Empidæ	5					*	1					*								*			*	*								*					*
Dolichopodidæ	5	*	*		*1	*2			*			*								*	*		*3	*	*						*	*					*
Phoridæ	4	*				*			*			*	*							*				*							*	*					*
Lonchopteridæ	4	*				*			*			*								*				*							*	*					*
Leptidæ	0	*				*		*6	*			*								*			*	*							*	*					*
Stratiomyidæ	3	*				*			*7			*					*	*		*			*	*		*		*	♂		*	*					?
Tabandidæ	3		*			*		*	*	*	*	*					*			*			*	*		*10		*			*	*					*
Bombylidæ	5		*		*	*			*			*						*		*				*							*	*					*
Cyrtidæ	0		*11																																			
Platypezidæ	4	*			*	*			*	*		*	*				*			*	*?			*		*				*?	*	*12					*
Pipunculidæ	4	*			*	*			*			*					*			*	*			*						*	*	*					*
Syrphidæ	4		*		*	*			*	*		*					*			*				*						*	*	*					*
Conopidæ	4		*		*	*			*	*		*			*		*			*				*						*	*13	*					*
Muscidæ	4	*	*		*	*	*		*	*		*			*		*			*				*						*	*	*					*

[1] In *Hybos*.
[2] Peculiar.
[3] In several species.
[4] Only in *Trineura*.
[5] In an atrophied condition in a few species.
[6] In *Leptis*.
[7] In *Hæmatopota* and other species of *Tabanus*.
[8] In *T. africanus* and *H. pluvialis*.
[9] In *Pangonia longirostris*.
[10] Small in *Comptosia occellata* New.
[11] Or aborted.
[12] Vestigial in several species.
[13] In some Acalyptrates.

the points of agreement are fairly strong in group 1, strong also in group 2, weak in groups 3 and 4, and fairly strong in group 5.

If the weak groups are examined anomalies will be seen in group 3; the Simulidæ seem out of place. I have lately found a structure in the palpi of *S. reptans* and *Rhyphus fenestralis* and *R. punctatus* ♀, which convince me of a near relationship and common ancestry of these two families. By adding *Simulium* to group 1 there results a larger proportion of similar characters.

TABLE III.

CHARACTERS OF THE GENITALIA IN THE NEMOCERA.

	Group.	Male.						Female.							
		Type of Penis.			Claspers Forcipes.			Ovipositor.			Appendages.			Receptacula.		
	Williston's Phyla.	Type 1.	Type 2.	Type 3.	Cercilike.	Simple Hamate.	Jointed Hamate.	Non-telescopic.	Short Telescopic.	Telescopic.	Cercilike.	Uncinate.	Styliform.	One.	Two.	Three.
Cecydomyidæ	1	*				*		*			*			*[1]		
Mycetophilidæ	1	*			*	*			*		*					
Bibionidæ	0	*			*			*			*					*
Scatopse	3	*				*		*			*			*		
Simulidæ	3	*			*			*			*			*		
Chironomidæ	2	*[2]				*		*			*				*[3]	
Psychodidæ	1	*				*		*					*			
Culicidæ	2		*			*		*				*				*
Ptychopteridæ	1			*		*		*				*				*
Erioptera	1							*				*				*
Tipulidæ	1	*				*		*				*				*
Rhyphidæ	1	*[4]			*			*			*			*		

[1] Where nothing is stated these organs are not chitinous and do not show in preparations.

[2] Approximates to that of the Rhyphidæ.

[3] 1–2 in *Ceratopogon*.

[4] Complicated and peculiar, but approximates.

Nor can I reconcile myself to the inclusion of the Scatopsinæ, though this subfamily, unlike the Simulidæ, will not fit well into group 1, notwithstanding that the genitalia show marked affinities with those of *Tipula*. The very marked specialization of the mouth structure and the three ocelli outweigh in importance the archaic eye structure, the bristle structure, especially that of the legs, and pharyngeal pump; I can not at present suggest

any change in the position they now occupy among the Bibionidæ, though I consider this family the most specialized of the Nemocera.

TABLE IV.

CHARACTERS OF THE GENITALIA IN THE BRACHYCERA AND CYCLORRAPHA.

	Group.	Male.						Female.								
		Type of Penis.			Claspers.			Ovipositor.			Appendages.			Receptacula.		
	Williston's Phyla.	Type 1.	Type 2.	Type 3.	Cerciliike.	Simple Hamate.	Jointed Hamate.	Non-telescopic.	Short Telescopic.	Telescopic.	Cerciliike.	Uncinate.	Styliform.	One.	Two.	Three or More.
Asilidæ	5	*				*		*			*	*			*	
Empidæ	5	*				*			*		*			*		
Dolichopodidæ	5	*				*				*		*		*1		
Phoridæ	4		*	*	*					*	*			*1		
Lonchopteridæ	4		?2	?	*			*			*				*3	
Leptidæ	0	*					*		*		*					*
Stratiomyidæ	3	*			*				*		*					*
Tabanidæ	3	*4					*	*			*					*
Bombylidæ	5	*			*			*			*					*
Cyrtidæ	0	*			*			*			*			?		
Platypezidæ	4	*			*	*			*		*				?	
Pipunculidæ	4	*				*		*					*			*
Syrphidæ	4			*	*	*				*	*	*				*
Conopidæ	4			*	*			*	*		*	*				*
Muscidæ	4			*		*		*	*	*	*	*	*	*	*	*

[1] Invisible in preparations.

[2] Indefinite, suggests affinities with *Dolichopus*.

[3] Transparent, only demonstratable by dissection.

[4] And theLeptidæ are nearer type 1 than 2 or 3. They only approximate.

If any reliance is to be placed on the genitalia, the Stratiomyidæ must belong in group 3, since the male type clearly connects the family with the Asilidæ, Empidæ and Dolichopodidæ. And the condition of the mentum sustains this view, though character of the leg pubescence is less decisive. The genitalia and venation of the Tabanidæ are so close that it seems impossible to separate the family; both find their place in group 5.

These points seem to show that group 3 is an artificial one; nor does group 4 inspire me with confidence, since I can not separate the Phoridæ and the Lonchopteridæ from the Asilidæ, Empididæ and Dolichopodidæ. Group 4 must be narrowed down to the Cyclorrhapha, and even here I think that I can trace the pedigree to group 5, if not to group 1.

I have now completed my analysis of the groups suggested by Williston from the antennal characters, so far as my imperfect data will permit, and I think that it shows that they furnish important hints as regards the phylogeny, particularly of the older flies, though the light they throw is intermittent and flickering. From their exposed position and frail structure the antennæ are subject, in the swiftest flying insects, to more than the usual risk of injury, which is possibly one reason for the extreme variation they show, ranging from three joints in the Scenopinidæ to thirty-nine in *Cerozodia*.

TABLE V.

STRUCTURE OF EYES IN NEMOCERA.

	Group.	Chitinous Structure.				Facets.			Pubescence.				Plates.[1]		Sexual Characters.				Ocelli.		
	Williston's Phyla.	Marked.	Lines Remaining.	Absent.	Double Eyes.	Circular.	Hexagons.	Two Sizes in ♂.	Much in ♂.	Less in ♀.	Equal ♂ ♀.	Absent.	Marked.	Simple.	Holoptic.	Dichoptic.	Equal.	Extreme Development.	Three.	Two.	Aborted.
Cecidomyidæ	1	*				*						*						*		**	*
Mycetophilidæ	1	*				*					*		*			*			*	*	
Bibionidæ: *Bibio* ♂	0		*		*		*	*	*					*	*				*		
Bibionidæ: *Bibio* ♀	0			*			*					*		*	*				*		
Bibionidæ: *Dilophus* ♂	0		*		*	*	*	*	*					*	*				*		
Bibionidæ: *Dilophus* ♀	0		*			*				*				*	*	*			*		
Bibionidæ: *Scatopse* ♂ ♀	3	*				*					*	*	*			*			*		
Simulidæ	3	*					*	*				*	*			*					*
Chironomidæ	2	*				*	*					*	*			*					*
Psychodidæ	1	*				*						*	*			*					*
Culicidæ: *Corethra*	2	*				*						*	*			*					*
Culicidæ: *Mochlonyx*	2		*			*						*	*			*					*
Culicidæ: *Culex*	2			*		*						*	*			*					*
Ptychoptera	1	*					*					*		*		*					*
Tipulidæ	1	*					*					*		*		*					*
Rhyphidæ	1		*				*				*			*		*			*		

[1] *Chironomus*.

[2] Exceptions exist.

To recapitulate, I think that group 1, with the inclusion of the Simulidæ, is a strong natural group, and even stronger tribe. Groups 3 and 4 fail altogether. Group 5 is fairly strong, but suggests the inclusion of the Stratiomyidæ, Xylophaginæ, Tabanidæ and Leptinæ, Lonchopteridæ and Phoridæ. As regards the Acanthomeridæ I have nothing to suggest.

TABLE VI.

STRUCTURE OF THE EYES IN BRACHYCERA AND CYCLORRHAPHA.

	Group.	Chitinous Structure.				Facets.			Pubescence.				Plates.		Sexual Characters.				Ocelli.		
	Williston's Phyla.	Marked.	Lines Remaining.	Absent.	Double Eyes.	Circular.	Hexagons.	Two Sizes in ♂.	Marked in ♂.	Less in ♀.	Equal ♂ ♀.	Absent.	Marked.	Simple.	Holoptic.	Dichoptic.	Equal.	Extreme Development.	Three.	Two.	Aborted.
Asilidæ	5	*					*	[1]			*	*		*		*			*		
Empidæ: *Clinocera*	5	*					*				*			*		*			*		
Empidæ: *Pachymera*	5		*				*					*		*		*			*		
Empidæ: *Hybos*	5			*			*					*		*			*	*	*		
Dolichopodidæ	5	*	*				[2]				*			*	*				*		
Phoridæ	4	*	*			*	[2]				*			*		*			*		
Lonchopteridæ	4	*					*					*		*		*			*		
Leptidæ	0	*					*	*			*	*		*	*				*		
Stratiomyidæ	3	*					*	*			*	*		*	*	*			*		
Tabanidæ: *Tabanus*	3	*					*	*	*					*	*						*
Tabanidæ: *Chrysops*	3		*				*			*	*	*		*	*				*		
Tabanidæ: *Hæmatopota*	3	*		♂			*	*	*	*				*	*						*
Tabanidæ: *Pangonia*	3	*		♂[3]			*	*				*		*	*				*		*
Bombylidæ	5	*					*				*	*		*	*				*		
Cyrtidæ	0						*				*	*		*					*		*
Oncodes	0	*					*					*		*			*	*		*	
Platypezidæ	4		*	*			*					*		*		*[4]			*		
Pipunculidæ	4			*			*					*		*	*				*		
Syrphidæ	4	*					*	*			*	*		*	*				*		
Conopidæ	4			*			*				*	*		*		*			*		
Muscidæ	4			*			*		*	*	*	*		*	*	*			*		*

[1] In *Leptogaster cylindrica* Deg. (exceptional) ♀.

[2] Often squares.

[3] The three stages have been found in the eyes of *H. pluvialis* in ♂.

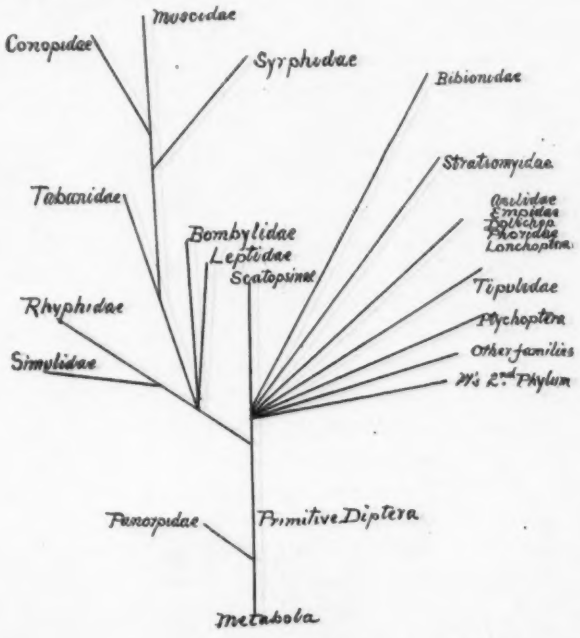
[4] *Opetia* exceptional.

With regard to the phylogeny of the Cyclorrhapha I have endeavored to show by a comparison of the mouthparts and venation, to which I may add the eye structure and general morphology, that the Tabanidæ stand close to the ancestral forms of the Cyclorrhapha, though the Syrphidæ and Conopidæ branched off when the insects had complete mouthparts and ocelli, long before the Muscidæ became the specialized and dominant group that it now is.

Williston says (page 332) that "every family save the Tipulidæ is, I believe, absolutely excluded from immediate genetic relations with the Brachycera, because of the venation and antennæ." I do not know of any anatomical structure that militates against

this view, and there are several that favor it; but I am strongly inclined to associate the Rhyphidæ with the Tipulidæ. The antennal characters and the venation are not insuperable objections; but I rely on the mouth, simple eyes and genitalia. The first of these characters is much less specialized than in *Tipula;* three ocelli are present, and the peculiar genitalia foreshadow those of the Tabanidæ and Leptidæ, while as I have already pointed out those of the Asilidæ, Stratiomyidæ and Dolichopodidæ and Empidæ seem to have been derived from a form like that of *Tipula.*

I venture to express these ideas in the shape of a tentative scheme, as follows:



If we are content to accept this view that the Rhyphidæ are close to the ancestral form of the Tabanidæ, we get another gleam of light on this obscure pedigree. If the idea is tested by the general morphology of the families it will be seen one section

is characterized by a comparatively large head, flat thorax and broad, flat abdomen; this includes the Rhyphidæ, Simulidæ, Tabanidæ and the Cyclorrhapha. The other section has a small head, humped thorax, and a narrow, depressed abdomen; this includes the Tipulidæ, and the Brachycera, except the Tabanidæ, Leptidæ, Therevidæ and Bombylidæ. The persistence of these two well-marked forms is good evidence of my argument, and, though it weakens the status of Williston's fifth phylum it establishes even more strongly his first and second.

On the Classification of the Nemocera.

If the table of the mouthparts of the older families is examined it will be seen that in only one family, the Cecidomyidæ, is the pharyngeal pump absent, but my specimens of this family are so few in number that probably this observation has no value. I feel confident that, with sufficient material, it will be demonstrated in some genera, even though vestigial. But, I am sure that *Bibio*, *Dilophus* and *Chironomus* do not have it, though it is present in *Scatopse* and some *Ceratopogones*. Not only are the Bibionidæ singular in this respect, but the eye structure, the limbs and bristle structure are all highly specialized. The usual coloration of certain species is singular and as remarkable as anything among Diptera, while the simpified venation and modified mouthparts mark them off as the most specialized family among the Nemocera. The only archaic characters remaining are the ocelli, the four-jointed palpi, and the long, membranous labium of *Dilophus*, with the palpi inserted near its end. In comparing the Chironomidæ with the Bibionidæ it will be seen that the mouth is less specialized, since the stipites and cardines are obvious and the mandibles are not fused, though embedded in the ventral side, as in *Bibio* and *Tipula;* the eyes are quite archaic in type, as is also the bristle and hair structure. Though the Culicinæ are highly specialized, the Corethrinæ are obviously older, and they possibly represent the continuance of an ancestral form of the Chironomidæ, as the venation is archaic and the mouth retains the pharyngeal pump.

These points show that the present arrangement of the families of the Nemocera, though convenient, is not founded on a true

principle. An arrangement which collocates those families possessing the most archaic characters would seem to be more proper. I would arrange Williston's groups 1 and 2 at the head of the Nemocera as follows:

1. Rhyphidæ.
2. Simulidæ.
3. Cecidomyidæ.
4. Mycetophilidæ.
5. Orphnephilidæ.
6. Psychodidæ.
7. Tipulidæ.
8. Dixidæ.
9. Culicidæ.
10. Blepharoceridæ.
11. Chironomidæ.
12. Bibionidæ.

I would place the Rhyphidæ first, since the venation is archaic, complete ocelli are present, the size is small and the pubescence and leg bristles are primitive. Add to these the structure of the mouthparts which appear to have their prototype in the Myriapoda, and it appears to me that the evidence is overwhelming that the family represents the most archaic type of Diptera; but I shall have more to say on this subject later.

The Simulidæ follow, as they also possess the peculiar structure of the palpi and have a nearly complete mouth-armature and are small in size. The Cecidomyidæ come next on account of the many-jointed antennæ and the archaic type of eye structure, but I admit that their place in the scheme is tentative. The Mycetophilidæ are difficult to separate from the Cecidomyidæ, but their eye structure, mouth, tibial bristles and the variable condition of the venation all justify their position here. The Orphnephilidæ are usually placed next to the Psychodidæ, which is a very old type retaining many archaic characters in eyes, mouth and wings.

The Tipulidæ follow as preserving the oldest type of venation, of leg pubescence and bristle-structure, together with the old types of eye structure, mouth and genitalia. The Dixidæ follow as the first family of Williston's second phylum. It is

difficult to say whether the Culicidæ or the Chironomidæ should have the precedence. The former are entitled to it on the venation (an obviously old form, possibly ancestral) and on the mouth, though I have a *Ceratopogon* in my cabinet with pharyngeal pump and broad-bladed maxillæ and mandibles, which is more primitive in type than the armature of *Culex*. But this is exceptional, the majority of the *Ceratopogones* being without mandibles and having the laciniæ of the maxillæ of a simpler type. The archaic type of eye structure is matched by *Corethra* and the absence of a pharyngeal pump in so many genera, whereas it is always present in the Culicidæ, decides in their favor.

The Blepharoceridæ follow, and the Chironomidæ after. I have already given my reasons for placing the Bibionidæ last.

Williston, in the true spirit of a paleontologist, has speculated on the primitive dipteron, and has given in words a reconstruction of a hypothetical form (p. 331, 2), as follows: "The primitive dipteron must have had eight fully developed longitudinal veins (including the auxiliary vein) with the second, third, fourth and fifth furcate, and a complete discal cell. The head was rather small, with the compound eyes separated equally by the front in both sexes. The ocelli were functional, and the maxillary palpi had four freely articulated joints; the labial palpi had probably already disappeared, though Weschè thinks differently. There were at least thirty-nine antennal joints in the male. The prothorax, mesothorax and metathorax were imperfectly fused, and the metanotum was visible from above. The abdomen had nine functional segments; the body was without differentiated bristles; and the tarsi had membranous pulvilli and empodia. The primitive flies were of moderate or small size, and probably crepuscular in habit, or at least denizens of shady forests."

Williston goes on to say that of modern Diptera the Tipulidæ approach most closely this hypothetical ancestor, principally in the venation, and remarks that they have become specialized by the almost complete loss of the ocelli, increase in size, and the loss of the pulvilli. He places the Rhyphidæ next in rank to the Tipulidæ. It seems to me, however, by his own diagnosis, that the Rhyphidæ are more primitive than the Tipulidæ. They

are small, and have functional ocelli and pulvilli. The mouth-parts are much less specialized (the mentum being developed) and they retain some remarkable archaic characters. I would protest against the time-honored custom of subjecting all other characters of Diptera to the venation.[1]

This reconstruction of the primitive dipteron by Williston has given me much pleasure and much food for thought, though I am not in agreement with him in his views of the labial palpi. I think that the primitive dipteron had, like all other contemporary insects, four palpi, and that they persisted in this condition until after the chief phyla had arisen, since a large majority of the Empidæ have what I believe to be undoubted labial palpi. I have no doubt that the structure of the labium in *Chrysops*, which I figured in 1904 in the cited paper on mouth-parts, shows aborted labial palpi, the palpigers. Savigny, in the dawn of orismology, pointed out remains on the labium of *Tabanus italicus*, and I can show a number of preparations in the same family with tufts of hair in similar situations to the palpigers of *Chrysops*.

Loss of Antennal Joints.

Williston discusses this subject on pages 328, 329 of the cited paper; some observations by myself may throw additional light upon it. I have in my cabinet a preparation of *Scatopse* of very small size, probably *S. minutissima* Verrall., in which the antennæ are unsymmetrical. The fourth and fifth joints are partially fused in the left antenna, the suture going only half through the segment; the right antenna has the full number nine of antennal joints, with the fourth and fifth separated (Figs. 1, 2); here we can clearly see that a middle joint has been lost. In preparations of *Dilophus* and *Bibio*, where, judging from the variations in number in different species, the antennæ are in an unstable condition I have several specimens where the distal joint consists of from three to six segments closely

[1] I will not quarrel with this conclusion, though I still think that holopticism outweighs in importance the archaic characters of ocelli and pulvilli and even of the mouth parts. It is quite evident, however, that the Rhyphidæ should no longer be placed at the extreme end of the Nemocera.—S. W. Williston.

joined together, the last or true terminal joint in an atrophied condition (Figs. 3, 4). It would thus seem that, in the antenna of *Dilophus* at least, joints may be lost either by fusion of the middle ones or the disappearance of distal ones.

The Character of the Hairs and Bristles on the Legs of Diptera and other Insects.

In the striking reconstruction of the primitive dipteron I have quoted, Williston has suggested that the body was without differentiated bristles. This character may well be extended to the limbs also. In 1902 I published some figures of the legs of diptera,[1] but these were mainly concerned with the strangest forms I could select; though the hairs and bristles were arranged in striking forms they were mostly subsidiary and depended on the altered shapes of the femora, tibiæ and tarsi. Later, in 1908,[2] I gave twelve figures of the microscopic appearance of preparations of legs taken from twelve different flies, three to illustrate a simple type, four the raptorial type, four the secondary sexual type, and one the parasitic type. The study of the limbs has led me to place considerable reliance on the hair and bristles as characters, and I find myself quite in agreement with Williston's idea that the simpler pubescence is the older form. My selection, a purely chance one, gave me as a result the legs of a tabanid, a leptid, and a stratiomyid as simple types. Going further back in an endeavor to realize what the primitive characters might be, I examined preparations of Myriopoda, *Blatta*, *Forficula* and *Panorpa*. These showed very wide differences in the bristles and hair with which they were more or less covered. Of the Myriopoda five species were examined, two Indian (Kashmir) and three British. A large *Scolopendra* is without pubescence, and with only two small bristles at the penultimate joint of the tarsi and two at the base of the claw. A species of *Scutigera* has an extraordinary number of tarsal joints (39) covered with short hairs, some of them short and stiff, with bristles at the larger joints, the parts that may represent the

[1] "Modifications of the Legs of Some Dipterous Insects." *Journal Queckett Club.*

[2] "On the Microscope as an Aid in the Study of Biology in Insects." *Journ. Royal Microscopic Society*, August, 1908.

coxæ, femora and tibiæ. Two British species (*Cryptops*) have short, stiff hairs regularly disposed over the legs; but a larger and broader species with a greater number of legs has them almost bare.

In *Blatta* a few short hairs are scattered over the limbs, but the femora and tibiæ are armed with many strong, sharp spines, which, in the genus *Phyllodromio*, are serrated with minute but regular barbs, undoubtedly specialized for raptorial purposes. In *Forficula* there are no bristles, only minute, soft scattered pubescence, which is much thicker on the inner side of the tarsi than elsewhere. In *Panorpa* a short, very even, uniformly long and regular pubescence is found studded with longer spines on the tibiæ and tarsi, and with tibial spurs of a curious and marked structure, each spur appearing as if it were made up of a number of fine hairs of various length, so that the edges appear almost plumose, certainly serrate.[1]

It may be of interest to record that *Peripatus novazealandiæ*, that remarkable survival, has neither pubescence nor bristles on its short forelegs or on any part of the skin, which, however, is studded with minute papillæ.

All these arthropods except *Peripatus* have one character in common, and that a very marked one. From the upper joints, or femora to the claw or claws, there is seen what under low magnification appears to be a thread-like tendon, but under high magnification a duct leading to the claw, either carrying poison to the claw or moistening the plate at the base of the empodium, and from that part the pulvilli. This duct might have been described from diptera instead of *Blatta*, *Forficula* or *Panorpa* so obvious are the homologies, but the arrangement of hair and bristles on the surface suggests no counterpart, except in *Panorpa*. A comparison of my preparations in the Nemocera with that

[1] In my paper on the systematic affinities of the Phoridæ in the *Transactions of the Entomological Society*, I stated that this structure was only to be found in the Mycetophilidæ and Phoridæ. I should have stated that only in the former family were they found in a size comparable and requiring a magnification of 250 diameters for elucidation. These on *Panorpa* and the diptera mentioned later are much larger and can be seen with lower powers, except in the case of the Rhyphidæ, which is a recent observation. Of course the presence of this structure in other insects admittedly of ancient type only strengthens my former argument, but it also shows the danger of dogmatic formulas.

insect shows that the pubescence of the legs approximated closely in *Gynoplistia bella*, particularly in the region of the tarsi; in *Ptychoptera albimana*, *P. lacustris*, *P. scutellaris*, and *Rhyphus fenestralis* the structure of the tibial bristles is practically identical. This is certainly remarkable, as Woodworth on the evidence of the venation[1] has suggested that this family is more closely related to the diptera than any other, branching off after the Neoptera had left the Metabola.

I think that we may assume that the primitive type of pubescence on the legs of diptera was somewhat similar to that which yet exists in *Rhyphus* and the Tipulidæ; and that when marked bristle structure or armature is found the insects are specialized. So we recognize, and this harmonizes well with other characters, that the Bibioninæ and the Culicinæ are the most specialized subfamilies among the Nemocera, such forms as *Dilophus*, or *Mucidus* and *Sabethes* making this clear. In the Mycetophilidæ, *Sciara* preserves the older type, while *Mycetophila* in the strong spines on the tibiæ and tarsi is more specialized, which idea is quite confirmed by the ocelli, three in *Sciara*, two in *Mycetophila*, and these remote from their usual position. The other families (I am not certain of the Blepharoceridæ) are all of the simpler types as is the genus *Scatopse* and the subfamily Corethrinæ.

Among the Brachycera, in the families with many genera, a number of variations between simplicity and complexity will be found, mostly as secondary sexual characters in the male, while the predaceous insects will be found modified in both sexes. The more striking examples of the latter will be found among the Empidæ, the Asilidæ appearing to confine their armature mostly to the tarsi. The Phoridæ have a peculiar and characteristic chætotaxy, but do not vary markedly; while the Leptidæ, Stratiomyidæ, Tabanidæ and Cyrtidæ are all of the simpler type, the Leptidæ most nearly like the Tipulidæ, retaining the peculiar bristle structure. The Platypezidæ and Pipunculidæ show various modifications of rows of long bristles or hairs as well as peculiarly modified bristles. In some Platypezidæ these bristles

[1] "Wingveins of Insects." Univ. California Publications, Entomology, Vol. 1, p. 145, 1906.

(as in *P. consobrina*) are, like the modified hind tarsi, found in both sexes.

Among the Cyclorrhapha, as might be expected, we find the characters of the legs extremely developed. Among the Syrphidæ, though there are many such simple forms as *Chilosia*, we find progressive degrees leading to great complexity, as in *Platycheirus* and *Pyrophæna*. Such a form as *Sphærophoria scripta* is an intermediate one; the general type is simple, but the under side of the middle femora of the male is studded with short sharp hairs absent in the female. The four genera of the Conopidæ examined all show a greater specialization than *Sphærophoria*. *Gastrophilus equi* has a long shaggy pubescence far removed from the simple forms. Of the Muscidæ alone a chapter might be written on the variations of the pubescence and bristles of the legs; and some flies, like *Glossina*, have structures which appear to be characteristic. All these modifications are those of strong bristles, though softer hairs are often present. I can not call to mind instances, unless it be *Calobata* where the pubescence is uniformly like that of *Gynoplistia* and the Panorpidæ, soft and weak.

On Certain Glands in the Mouths of Some Myriopoda and Diptera.

In the limb-like maxillæ of *Scutigera* (a centipede with compound eyes) there are organs of striking structure. In addition to the poison glands, which may easily be mistaken for tendons or overlooked, there are transparent chitinous bulbs communicating with apertures in the claws by ducts of moderate length. These bulbs are studded with a number of short tubular processes which show clearly when the edges of the organs are focused. These are peculiar structures of characteristic appearance, and are very unlikely to be confounded with other organs. I naturally reached the conclusion that these were poison glands, as the bite of the centipede, in addition to the punctures of the claws, is known to be poisonous. With this idea I was surprised to find in the maxillary palpi of *Rhyphus fenestralis* ♀ a similar structure which I have figured in the cited paper on mouth-parts, as sense organs. These communicate with the air by fairly large openings

in the walls of the palpi (Fig. 14). Unfortunately I have no preparation of the male of this species, but I find a similar structure in the male of *R. punctatus*, though smaller in size and with a shorter duct; as also in a Tasmanian species as well developed as in the former species. The differences between these two species in this respect are so marked that it is possible to separate them on this character alone (Fig. 13).

The uses of the organ are obscure, but they are probably similar in both the myriopod and the insects. The poison duct in the legs is quite similar to that part in the maxilla; it traverses many joints and opens underneath the claw between two bristles inserted at its base. Without a doubt it is the homologue of the duct which moistens the pulvilli in the flies. Again referring to the maxillary gland, my observations have not ended here, as the palpi of *Simulium reptans* ♀ and *S. ornatum* ♂ have similar structures, though they communicate with the air by a different opening. Moreover in the mouth of two British species of *Cryptops*, on the maxillæ, or more properly speaking the maxillipeds, and absolutely homologous in situation and structure, are similar glands to those found in the Indian *Scutigera*.[1]

That this structure should be found surviving in Diptera is exceedingly remarkable, but not more so than the fact that the duct which leads the poison to the many claws of *Scoliopendra* should be found in a precisely similar condition in nearly all insects. I have found it throughout Diptera, in *Blatta*, *Forficula*, *Panorpa*, Lepidoptera, Hemiptera, in fact in all insects where there are membranes on the claws that need irrigation.

I have studied the sense organs of insects for many years, and by comparing the large number of preparations, using modern optical methods and objectives I have become familiar with their appearance in the antennæ, palpi and mouths of Diptera, and many other insects. Unless I am greatly mistaken, and mistakes are easily made in such minute structures, I can say with confidence that there are found in the palpi of *Rhyphus* and *Simulium* homologous organs of peculiar structure; and judging from a comparison with *Scutigera*, this character is one of the

[1] I find the structure in a modified form in a small *Lithobius*, found in a garden in London.

most ancient hitherto observed in Diptera, a character which existed before there were winged insects, and consequently before venation, a character which is quite in agreement with the idea that the Rhyphidæ are among the most archaic types, the least specialized of all flies.

Summary of New Observations.

1. Ankylosis of middle joints of antennæ of *Scatopse*.
2. Fused distal joints in the antennæ of *Dilophus*.
3. Observations on the legs of myriapods and insects.
4. The tibial bristles of *Panorpa*, the Rhyphidæ, Mycetophilidæ, Tipulidæ, Leptidæ and Phoridæ are all of the same peculiar structure, and, excluding the Phoridæ, the general pubescence of the legs is approximately similar.
5. A peculiar structure exists in the mouths of some Myriapoda, and similar structures in the palpi of the Rhyphidæ and Simulidæ.
6. The pharyngeal pump has been found in the heads of the Rhyphidæ, Psychodidæ, a *Ceratopogon*, *Scatopse* and the Simulidæ; and it has been dissected out in a vestigial condition from the heads of *Hæmatopota pluvialis* and *Tabanus africanus* ♀.
7. A *Ceratopogon* with mandibles has been found.
8. An observation on the number of receptacula of *Lonchoptera flavicauda* is recorded.
9. A suggestion is offered that the peculiar genitalia of the Rhyphidæ have some affinity with those of the Tabanidæ and Leptidæ.
10. The mentum is fully developed in the Rhyphidæ, another important link connecting the Nemocera with the Brachycera.

Explanation of Plate.

Fig. 1. Di gram of left antenna of *Scatopse minutissima*, to show the partial ankylosis between fourth and fifth joints.

Fig. 2. Diagram of the right antenna of same insect, showing normal structure.

Fig. 3. Diagram of antenna of *Dilophus febrilis*, showing condition of distal joint.

Fig. 4. Diagram of antenna of *Dilophus albipennis* to show condition of distal joint.

Fig. 5. Gland (?) from the maxilla of *Scutigera*, highly magnified. Its situation is shown in Fig. 12*a*.

Fig. 6. Second tarsal joint of hindleg of *Gynoplistia bella* (Tipulidæ) to show pubescence under magnification of 60 diameters.

Fig. 7. Second tarsal joint of *Leptis scolopacea* under like magnification.

Fig. 8. Tibial bristle of hindleg of *Panorpa communis*. 60 diameters.

Fig. 9. Tibial bristle of hindleg of *L. scolopacea*.

Fig. 10. Tibial bristle of hindleg of *G. bella*.

Fig. 11. Second tarsal joint of hindleg of *Panorpa communis*, as seen with magnification of 60 diameters.

Fig. 12. Diagram of a segment from the mouth of *Scutigera* to show the maxillæ and situation of the poison (*b*) and other glands (*a*).

Fig. 13. Second joint of maxillary palpus of *Rhyphus punctatus*. Diagram in optical section to show gland (*a*) with duct opening in the anterior portion of the joint, and the sense organ which is probably olfactory (*b*), as seen with magnification of 300 diameters.

Fig. 14. Trophi of *Rhyphus fenestralis* showing the ventral side. The right palpus shows the sensory structure on its surface, while the left is drawn in optical section to show shape, situation and structure of the gland contained in interior. The mentum shows a distinct median structure, and below it are the submentum. and a portion of the pharyngeal pump. *m*, mentum; *sm*, submentum; *pp*, pharyngeal pump; *a*, gland; *l*, lacinia of maxilla. Drawn from several specimens mounted with and without pressure, showing structure under magnification of 300 diameters.

Fig. 15. Hypopharynx of *R. fenestralis* showing submentum, an unusual condition.

Fig. 16. Labrum of *R. fenestralis*.

Fig. 17. Second joint of palpus of *R. brevis* Walker, differing from *R. punctatus* in the size and attachment of the gland (*a*) and the character of the sense organ (*b*) and from *R. fenestralis* in the attachment of the gland to the wall of the segment, and in the character of the sense organ. Same magnification as Fig. 13.

Fig. 18. Tibial bristle of hindleg of *R. fenestralis*. Magnification of 300 diameters.

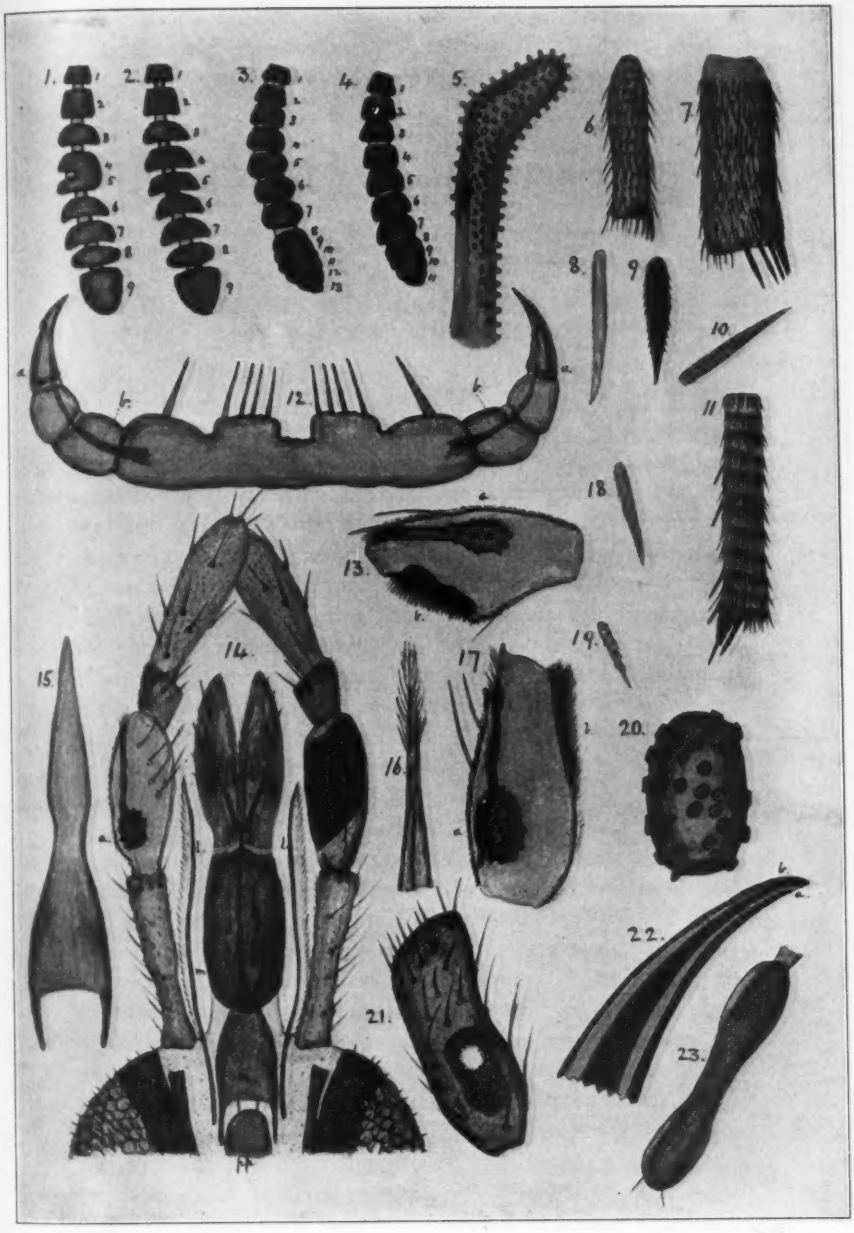
Fig. 19. Tibial bristle of hindleg of *R. punctatus*. Same magnification.

Fig. 20. Gland from interior of second joint of palpus of *Simulium reptans*, to show structure on side opposite the opening.

Fig. 21. Second joint of palpus of *S. reptans*, highly magnified.

Fig. 22. Point of maxilla of *Scutigera*, seen in optical section and highly magnified, showing openings of the poison duct (*a*) and of other gland (*b*).

N.B. The size of the figures has no relative significance.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